Institute of Pharmaceutical Chemistry
University of Szeged

SYNTHESIS AND STRUCTURE OF (BI)CYCLOALKANE-FUSED HETEROCYCLES. PHARMACOKINETIC STUDY OF A NEW ANXIOLYTIC

Ph.D. thesis

by

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Publications related to the thesis

1. G. Stájer, A. E. Szabó, **J. Szúnyog**, G. Bernáth, P. Sohár:
   Synthese von gesättigten methylen-überbrückten 1,3-Benzoxazinen
   Chem. Ber. **117**, 3205-3210 (1984); *Magy. Kém. Foly.* **90**, 544 (1984)

2. P. Sohár, G. Stájer, A. E. Szabó, F. Fülöp, **J. Szúnyog**, G. Bernáth:
   Preparation and systematic NMR spectroscopic study of norbornane/norbornene condensed 2-arylimino-1,3-oxazines and thiazines
   J. Chem. Soc., Perkin **2**, 599-605 (1987)

3. P. Sohár, G. Stájer, I. Pelczer, A. E. Szabó, **J. Szúnyog**, G. Bernáth:
   Synthesis and NMR study of norbornane/norbornene-fused tetracyclic azetidinones
   Tetrahedron **41**, 1721-1732 (1985)

4. G. Stájer, F. Csende, G. Bernáth, P. Sohár, **J. Szúnyog**:
   Preparation and steric structure of 3(2H)-pyridazinones and 1,2-oxazine-6-ones fused with three- to six-membered saturated carbocycles or norbornane skeleton
   Monatsh. Chem. **125**, 933-944 (1994)

5. F. Miklós, F. Csende, G. Stájer, P. Sohár, R. Sillanpää, G. Bernáth, **J. Szúnyog**:
   Synthesis and structure of methanobenzocyclooctene derivatives
   Acta Chem. Scand. **52**, 322-327 (1998)

6. P. Sohár, G. Stájer, A. E. Szabó, **J. Szúnyog**, G. Bernáth:
   Preparation and structure of partially saturated isoindolo[1,2-*b*]- and -[2,1-*a*]quinazolines
   Heterocycles **48**, 175-180 (1998)

7. G. Stájer, A. E. Szabó, P. Sohár, **J. Szúnyog**, G. Bernáth:
   The first preparation of a heterotricycle, [1,3]oxazino[2,3-*a*]isoindole-2,6-dione, by a retro Diels-Alder method
   Synthesis **1999**, 718-720

8. **J. Szúnyog**, I. Hazai, Gy. Grézal, I. Klebovich:
   Comparative bioanalytical study of ^{3}H-deramciclane (EGIS-3886) in dog plasma, using a gas chromatography-nitrogen-selective detection (GC-NPD), a new GC-radiochemical detection (GC-RD) and a liquid scintillation method
   Chromatogr. **48**, 133-139 (1998)

Contents

Introduction

Most organic drug compounds are aromatic heterocycles; their systematic study and manufacturing started at the beginning of the 20th century. It is interesting that the preparation of the saturated heterocyclic derivatives began only fifty years later. Clarification of the theoretical background of stereoisomerism and investigation of the stereoisomers of saturated molecules commenced only after the appropriate analytical equipment became available.

One of the advantages of the use of saturated heterocycles as drug compounds is that the heteroaromatic compounds and their biotransformation products are sometimes toxic. The saturated analogues which were not investigated until the 1970s, and especially those which contain heteroatoms in the 1,3 positions, have essentially lower toxicity.[9] Since 1979, Bernáth and his co-workers have been working on the synthesis and conformational analysis of fused-skeleton fully or partly saturated heterocyclic compounds at the Institute of Pharmaceutical Chemistry at the University of Szeged.

The syntheses of the saturated analógues of condensed aromatic compounds do not simply involve application of the reactions characteristic of the aromatic compounds. The saturated heterocycles can frequently be prepared by different methods and they mostly react in different ways. On cyclization, new chiral centres are formed on the saturated sp^3 carbon atoms. Hence, for the preparation of structural isomers, *e.g.* stereohomogeneous *cis* and *trans*, *exo* and *endo* compounds, either stereoselective reactions have to be applied, or the stereoisomers formed (mostly diastereomers) have to be separated by appropriate methods.

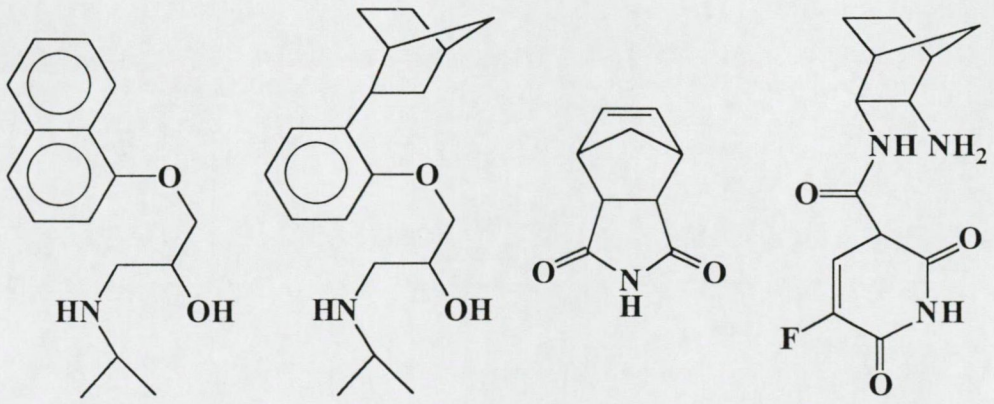
We have recently often used *cis* and *trans*, *exo* and *endo* starting materials, or separated the isomeric compounds in the early stages of preparation. In the course of our work, when possible, we applied sterically controlled reactions and separated the resulting isomers by chromatographic methods, which resulted in stereohomogeneous *cis* and *trans*, *exo* and *endo* products. The structures of the isomers (conformation and configuration) were established by IR and NMR spectroscopy, or X-ray methods were also used for structure elucidation. On the basis of these investigations, correlations could be established between the stereostructure and the pharmacological activity.

Literature review

The preparations of *cis*-trimethylene-, *cis*- and *trans*-tetramethylene- or pentamethylene-dihydro-1,3-oxazines,[10] the analogous tetrahydro-1,3-oxazines,[11,12] 1,3-oxazine-2-ones,[13,14] 1,3-oxazine-2-thiones,[15,16] 1,3-oxazine-4-ones[17,18] and related condensed heterocyclic compounds, such as the pyrimidone derivatives,[19] have been published from the Institute. The stereostructures of these compounds were established by means of ^{1}H and ^{13}C NMR spectroscopy and in some cases X-ray diffraction studies.[10,15,20] The homologous *cis* and *trans* isomers mentioned above were synthesized for systematic pharmacological study.

In the course of the research on fused 1,3-oxazine derivatives, numerous 1,3-oxazine derivatives containing a norbornane or norbornene skeleton were prepared.[21-25] Although the norbornane moiety itself is rarely found in natural compounds, its trimethyl derivatives are common, for isoprene (containing five carbon atoms) is a starting unit in the biosynthesis of mono and higher terpenes.

The norbornane skeleton often occurs among synthetic drugs. A review published in 1983 describes hundreds of norbornane derivatives as therapeutically active compounds,[26] *e.g.* the Propranolol analogue β-receptor-blocker Bornaprolol[27] (Scheme 1), which acts for a long time because of the slow dissociation of the drug complex.[28] Noreximide is a stimulant of the central nervous system.[29] Some of the 5-fluorouracil derivatives, *e.g.* 1-[*N*-(3-amino-2-norbornyl)carbamoyl]-5-fluorouracil, have an anticarcinogenic effect.[30] Norbornane derivatives

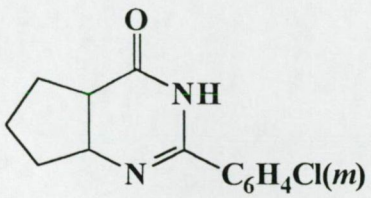


Propranolol (Stobetin®) | Bornaprolol | Noreximide (*diexo-diendo* mixture) | 1-[*N*-(3-amino-2-norbornyl)-carbamoyl]-5-fluorouracil

Scheme 1

are used not only as potential pharmacons, but also as herbicides, insecticides, *etc.*

One of the reasons for my work was that 2-*m*-chlorophenyl-*cis*-5,6-trimethylene-4-pyrimidone, synthesized by Bernáth *et al.*, exhibits outstanding analgetic and anti-inflammatory activity, and was chosen by Chinoin Pharmaceutical Co. under the notation CHINOIN 143[31] for further development (Scheme 2).



CHINOIN 143
G. Bernáth, L. Gera, Gy. Göndös, M. Hermann, M. Szentiványi, Z. Ecsery, E. Janvári, Ger. Pat. 2643384 (1977); ref. *Chem. Abstr.* **87**, 168078b (1977)

Scheme 2

It could be expected that the norbornane and pyrimidinone moieties together would give an effective structure, especially when the annelation is *cis*.

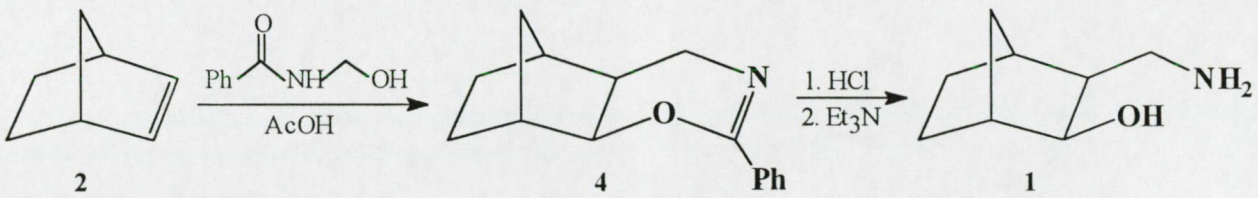
Stájer *et al.* have synthesized a large number of norbornane- and norbornene-condensed heterocycles for pharmacological purposes. These are ethylene-bridged or ethenylene-bridged derivatives of the trimethylene-condensed heterocycles in their cyclopentane ring.[21a, 25] Two isomeric series of these compounds containing rigid carbobicycles have been prepared: the *endo-endo* and *exo-exo* derivatives.

I joined in the work on the preparation of pharmacologically active compounds in 1981. Initially, I took part in the synthesis and structure establishment of 1,3-oxazines, and dealt later with the separation of isomers obtained on a semipreparative scale by HPLC. Recently, I have been working at EGIS Pharmaceutical Co. on pharmacological and pharmacokinetic studies of compounds chosen, *e.g.* deramciclane, which contains a norbornane skeleton, and have participated in the structure investigation of its metabolites.

Results

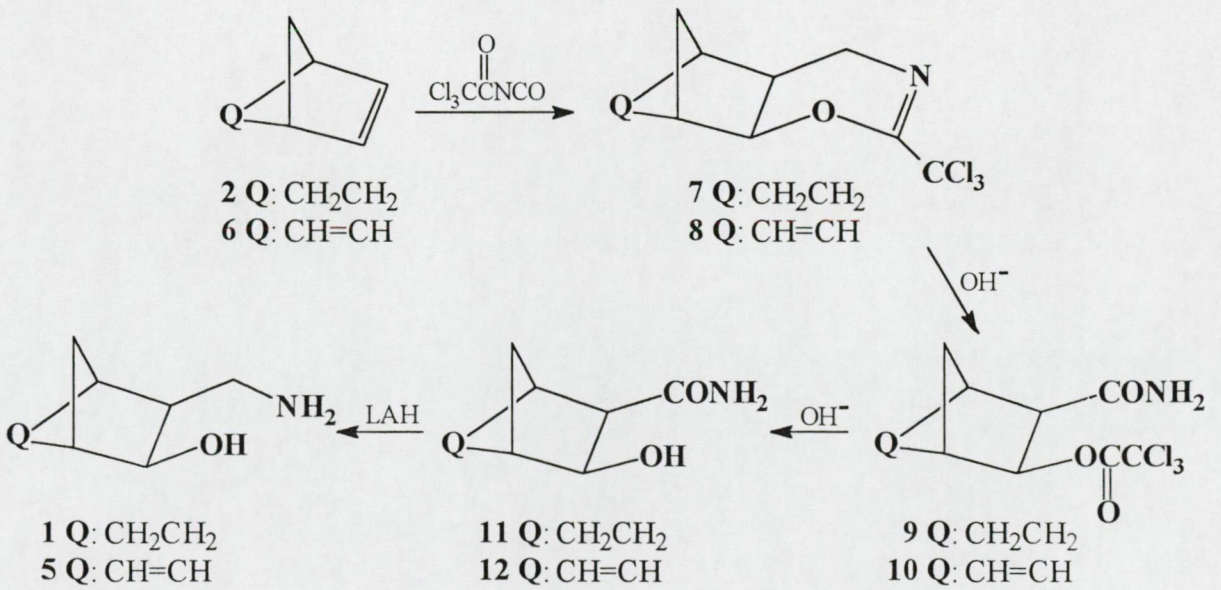
1. Synthesis and conformational study of partially saturated 1,3-benzoxazines, 1,3-benzoxazine-2-ones and 1,3-benzoxazine-2-thiones[1]

The starting aminoalcohol (**1**) was prepared by acidic hydrolysis of the cycloadduct of norbornene (**2**) and *N*-hydroxymethylbenzamide.[32-34] The yield of 2-*exo*-hydroxy-3-*exo*-aminomethylbicyclo[2.2.1]heptane (**1**) was low, however: 15%.



Scheme 3

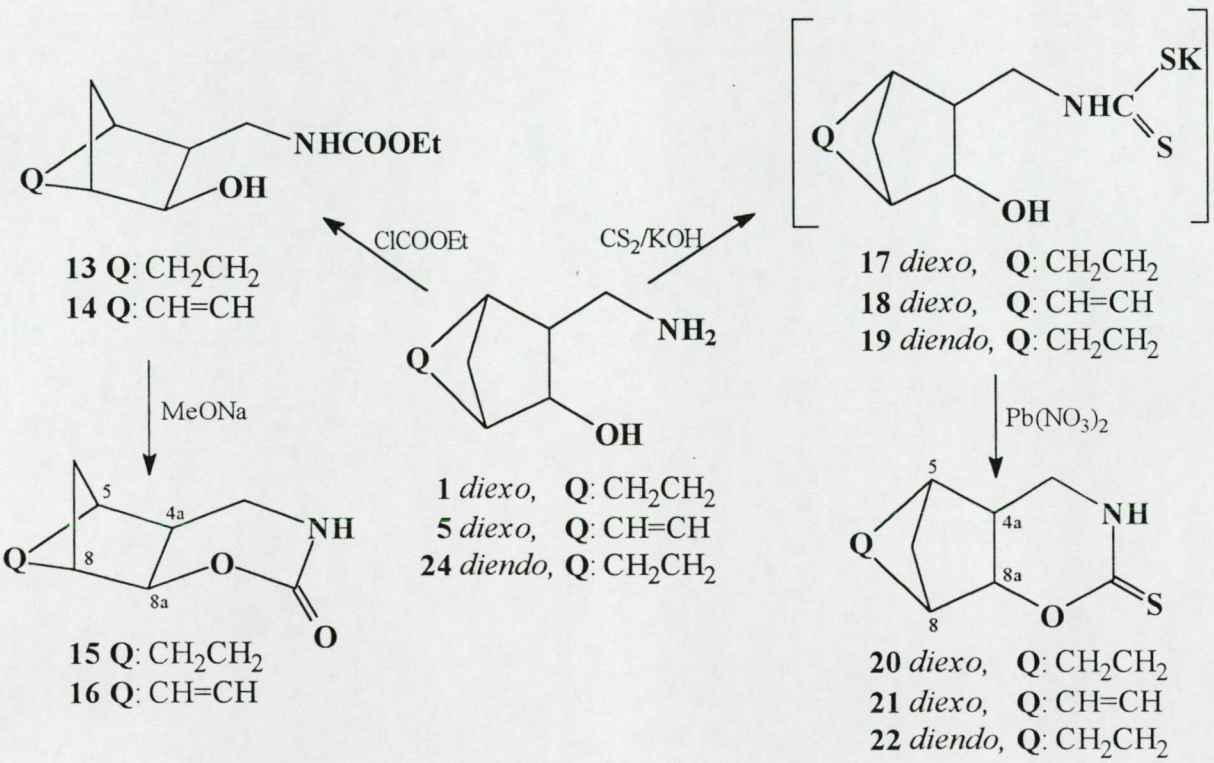
For a higher yield and to prepare the unsaturated aminoalcohol (**5**), trichloroacetyl isocyanate was used[34] (Scheme 4). Hydrolysis[34] of 2-trichloromethyl-5,8-methano-4a*r*,5*c*,6,7,8*c*,8a*c*-hexahydro-4*H*-1,3-benzoxazine-4-one (**7**) and 2-trichloromethyl-5,8-methano-4a*r*,5*c*,8*c*,8a*c*-tetrahydro-4*H*-1,3-benzoxazine-4-one (**8**) gave the trichloroacetyl-β-hydroxy-carboxamides **9** and **10.** After removal of the trichloroacetyl group, these furnished



Scheme 4

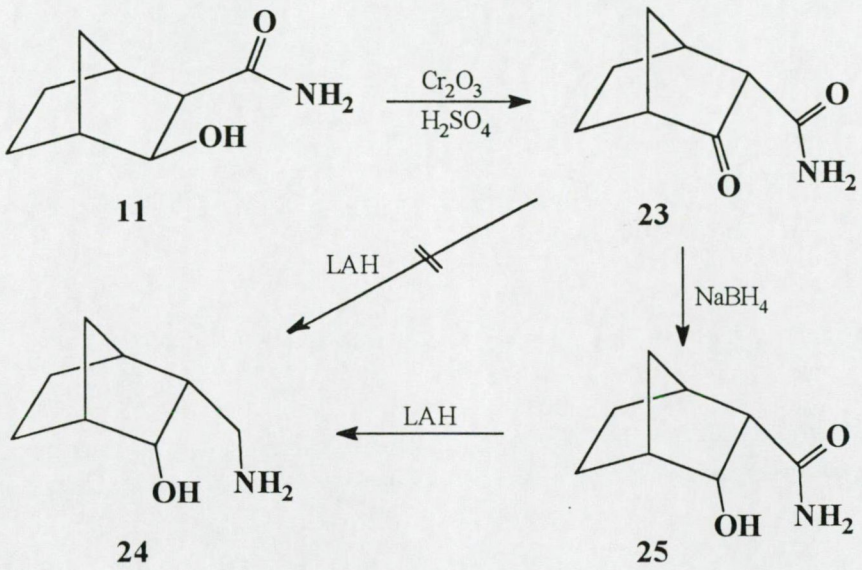
the saturated and unsaturated 2-hydroxy-1-carboxamides (**11** and **12**) which were reduced with lithium tetrahydridoaluminate(III) (LAH) to **1** and **5**; in this way, 40-45% aminoalcohols were obtained.

From **1** and **5,** the carbamates **13** and **14** were prepared with ethyl chloroformate, and were cyclized to 5,8-methano-4a*r*,5*c*,6,7,8*c*,8a*c*-hexahydro-4*H*-1,3-benzoxazine-2(3*H*)-one (**15**) and 5,8-methano-4a*r*,5*c*,8*c*,8a*c*-tetrahydro-4*H*-1,3-benzoxazine-2(3*H*)-one (**16**) (Scheme 5). The thiones **20-22** were prepared from **1**, **5** and **24** through the dithiocarbamates **17-19** (not isolated) by cyclization with Pb(II).



Scheme 5

On oxidation of carboxamide **11** with chromic acid[35, 36], the β-oxo-carboxamide **23** was obtained (Scheme 6), which was reduced to the *diendo*-β-hydroxycarboxamide **25** with $NaBH_4$. Direct reduction of the carboxamide **23** to the stereohomogeneous aminoalcohol **24** with LAH was not successful because the 1:1 mixture of *diendo-diexo* derivatives was obtained. However, reduction of the β-hydroxycarboxamide **25** with LAH gave the stereo-homogeneous *diendo*-aminoalcohol (**24**).



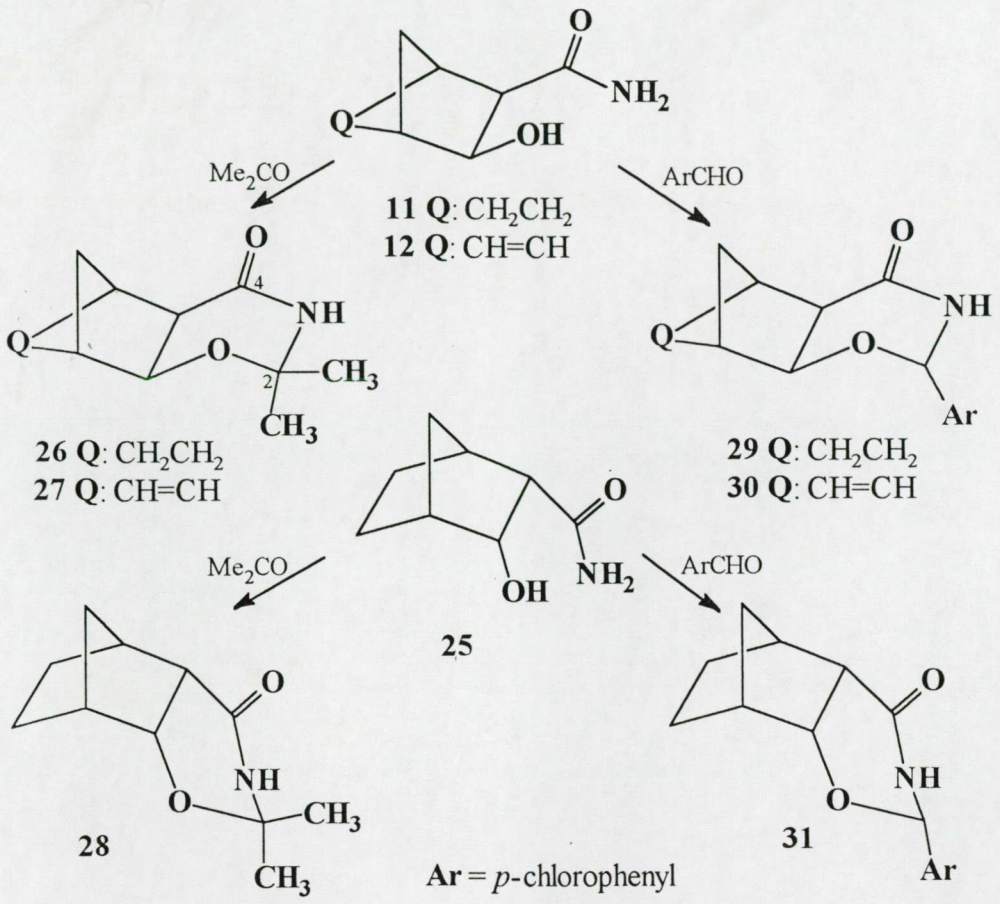
Scheme 6

2,2-Dimethyl-5,8-methano-4a*r*,5*c*,6,7,8*c*,8a*c*-hexahydro-2*H*-1,3-benzoxazine-4(3*H*)-one (**26**), 2,2-dimethyl-5,8-methano-4a*r*,5*c*,8*c*,8a-tetrahydro-2*H*-1,3-benzoxazine-4(3*H*)-one (**27**) and 2,2-dimethyl-5,8-methano-4a*r*,5*t*,6,7,8*t*,8a*c*-hexahydro-2*H*-1,3-benzoxazine-4(3*H*)-one (**28**) were obtained by cyclization of the 2-hydroxy carboxamides (**11**, **12** and **25**) with acetone (Scheme 7). 2-*p*-Chlorophenyl-4a*r*,5*c*,6,7,8*c*,8a*c*-hexahydro-1,3-benzoxazine-4(3*H*)-one (**29**), 2-*p*-chlorophenyl-4a*r*,5*c*,8*c*,8a*c*-tetrahydro-2*H*-1,3-benzoxazine-4(3*H*)-one (**30**) and 2-*p*-chlorophenyl-4a*r*,5*t*,6,7,8*t*,8a*c*-hexahydro-2*H*-1,3-benzoxazine-4(3*H*)-one (**31**) were prepared from **11**, **12** and **25** with aldehyde.

From the reactions of the aminoalcohols **1** and **24** with imidates, 2-aryl-5,8-methano-4a*r*,5*c*,6,7,8*c*,8a*c*-hexahydro-4*H*-1,3-benzoxazines (**32a-d**) and 2-aryl-5,8-methano-4a*r*,5*t*,6,7,8*t*,8a*c*-hexahydro-4*H*-1,3-benzoxazines (**33a-b**) were formed, the 2-phenyl derivative of which (**32a**) was already known[32] (Scheme 8).

The 2-aryl-5,8-methano-4a*r*,5*c*,8*c*,8a*c*-tetrahydro-4*H*-1,3-benzoxazines (**34a-d**) were prepared from norbornadiene **6** by cycloaddition of *N*-hydroxymethylbenzamides (Scheme 9).

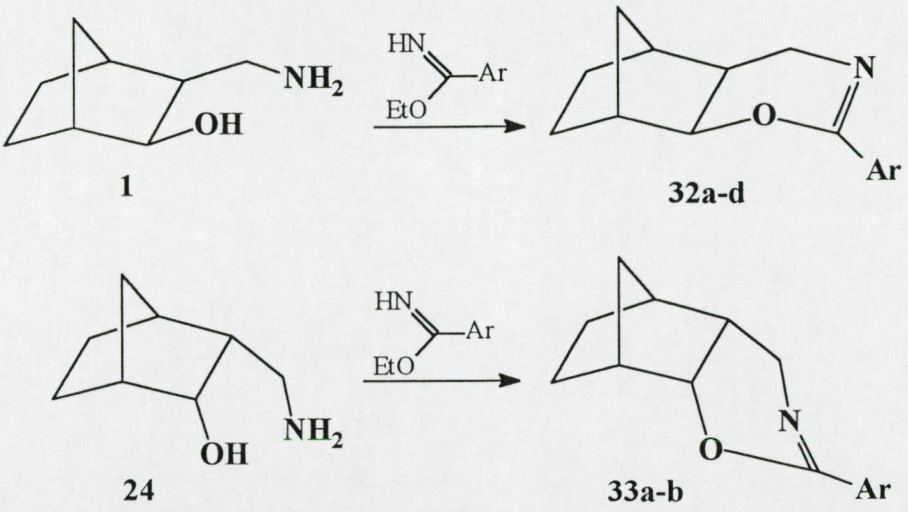
In the IR spectra, the carbonyl lines for **15** and **16** were found at 1720-1695 cm^{-1}. The νNH bands appear in the region 3450-3170 cm^{-1}; the analogous thiocarbonyl lines for **20-22**



Scheme 7

lie in the interval 1590-1540 cm^{-1}, and the νNH bands at 3200-3150 cm^{-1}. The amide-I bands of the derivatives **26-31** were found at 1650-1645 cm^{-1}, while the νNH lines appeared in the region 3750-3350 cm^{-1}. The characteristic νNH lines were missing from the spectra of **32a-d**, **33a-b** and **34a-d**; instead, the νN=H lines of a cyclic iminoether could be identified at 1650-1640 cm^{-1}.

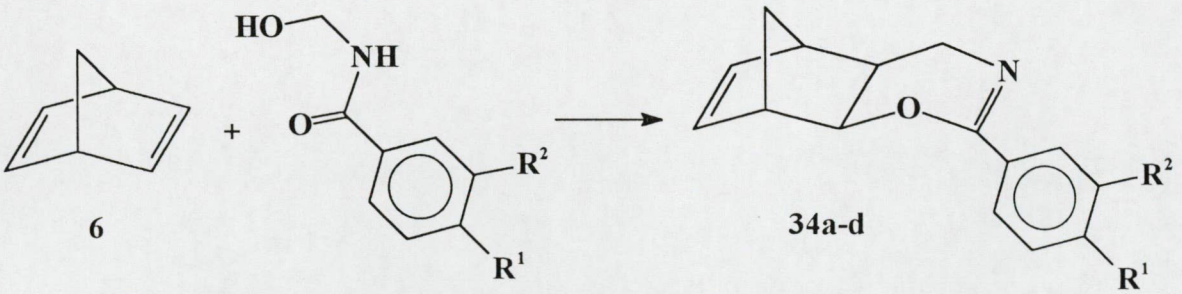
The annelation of the norbornane moiety and the heterocycle was established by the multiplicity of the H-8a proton resonance signal. The dihedral angle H-8–C-8–C-8a–H-8a in the *diendo* compounds **21, 28, 31** and **33a-b** is ~50°, while for the *diexo* analogues it is ~90°. Thus, for the *diendo* compounds, the spin-spin coupling of H-8,8a causes a significant



a: C_6H_5; **b**: $C_6H_4Cl(p)$; **c**: $C_6H_4Cl(m)$; **d**: $C_6H_4CH_3(p)$

Scheme 8

splitting, whereas in the spectra of the *diexo* compounds the magnitude of this splitting is smaller than 1 Hz.[37] The H-8a signal in the spectra of the *diexo* compounds **15, 16, 20, 21, 26, 27, 29, 30, 32a-d** and **34a-d** is a doublet with J = 8 Hz, corresponding to the H-4a,8a coupling, while in the spectra of the *diendo* compounds it appears as a double doublet with J = 9.5 and 4.5 Hz; the H-8,8a coupling causes a splitting of about 4 Hz.



a: $R^1 = R^2 = H$; **b**: $R^1 = Cl$, $R^2 = H$; **c**: $R^1 = H$, $R^2 = Cl$; **d**: $R^1 = CH_3$, $R^2 = H$

Scheme 9

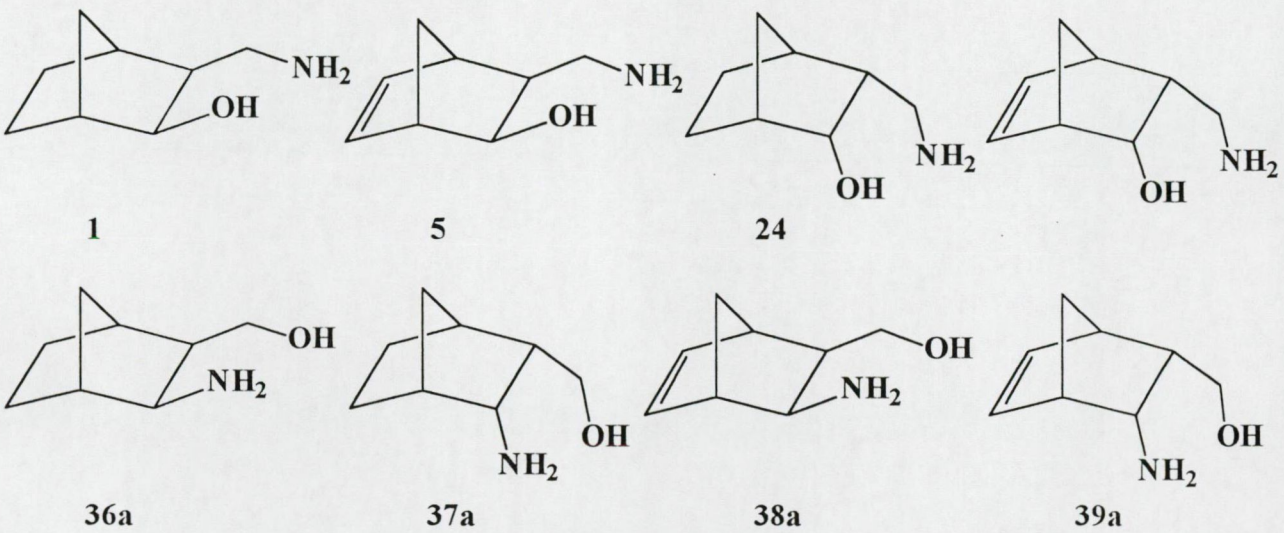
There are significant differences in the H-5 and H-8 chemical shifts of the saturated and unsaturated compounds. In the latter group, the *-I* effect of the C=C group causes a downfield shift: the H-5 signal is in the region 2.5-2.9 ppm, while that of H-8 lies at 2.54-3.25 ppm. For the norbornanes, the two shift intervals are 2.05-2.25 and 2.05-2.7 ppm, respectively.

The signals of the aromatic protons in the ^{1}H NMR spectra appear at 7.2-7.9 ppm, with the expected multiplicity and intensity, while there are no proton signals in this region if the aromatic substituent is missing.

The C-2 signals in the ^{13}C NMR spectra are characteristic of the different functional groups. The signals of the carbonyl atom C-4 in **26-31** are in the region 170-172 ppm, as expected.

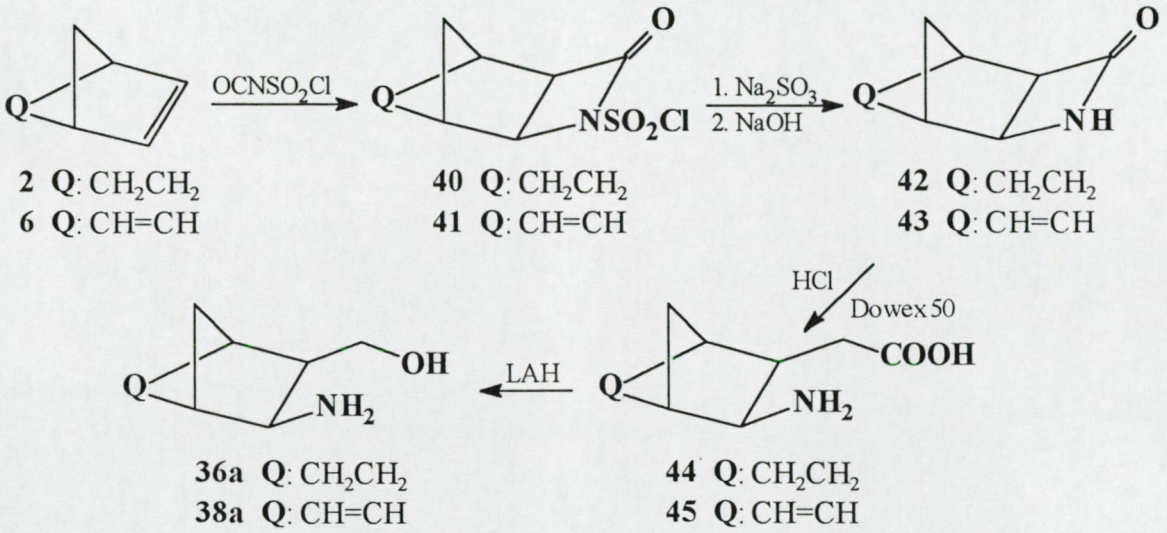
2. Preparation and NMR study of norbornane- and norbornene-fused 2-phenylimino-1,3-oxazines and -thiazines[2]

As concerns the starting 1,3-aminoalcohols, seven of the possible eight (**1**, **5**, **24** and **36a-39a**) were synthesized; these differ as regards the double bond of the norbornane moiety and the positions of the oxygen and nitrogen atoms; three of them (**1**, **5** and **24**) were described earlier[1, 21] (Scheme 10).



Scheme 10

The *diexo*-aminoalcohols **36a** and **38a** were prepared by the cycloaddition[38] of norbornene **2** or norbornadiene **6** and *N*-chlorosulphonyl isocyanate (Scheme 11). The *N*-chlorosulphonylazetidinones **40** and **41** were reduced with sulphite[39] and the 3-*exo*-4-azaoxo-cyclo[4.2.1.$0^{2,5}$]nonane (**42**) and -non-7-ene (**43**) formed were hydrolysed with HCl.[38] After purification on an ion-exchange resin, the hydrochlorides of the saturated (**44**) and the unsaturated (**45**) bicyclic amino acids were obtained.[40] By reduction with LAH,

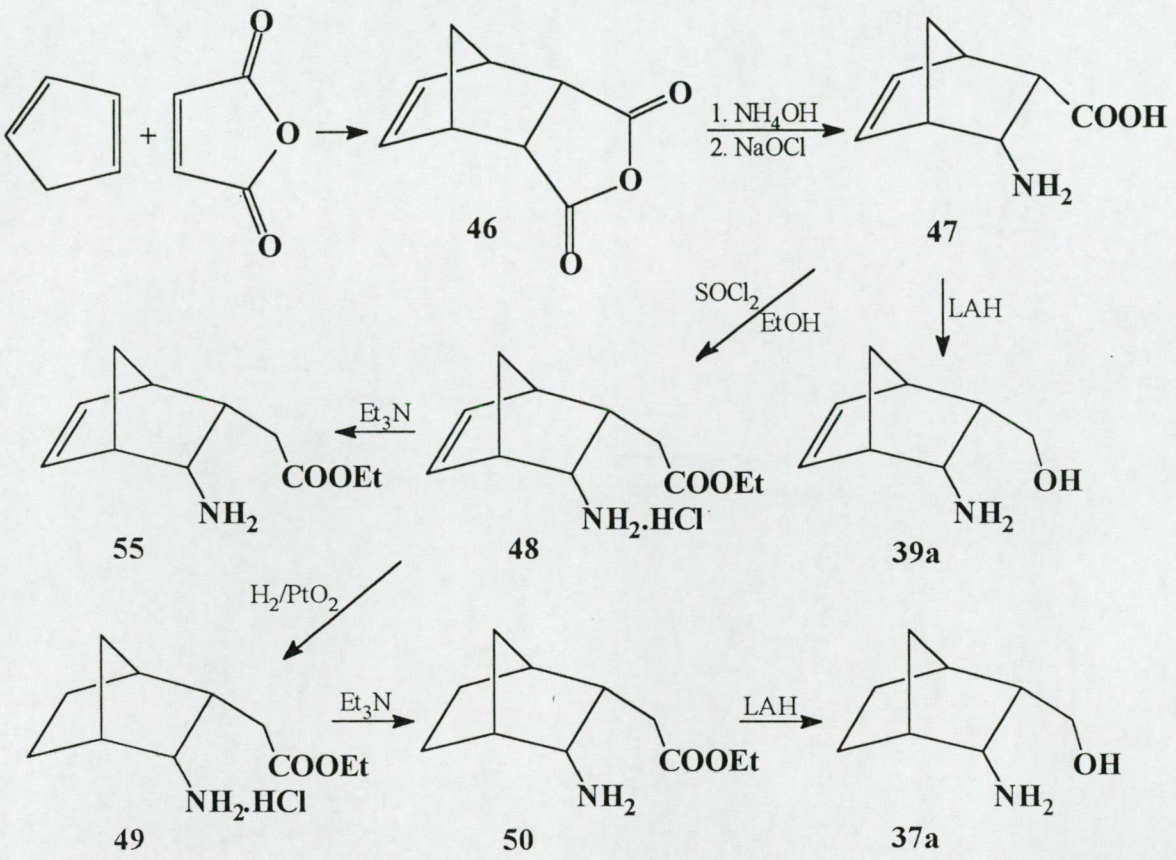


Scheme 11

3-*exo*-hydroxymethylbicyclo[2.2.1]heptyl-2-*exo*-amine (**36a**) and 3-*exo*-hydroxymethylbicyclo[2.2.1]hept-5-enyl-2-*exo*-amine (**38a**) were prepared.

The *diendo* analogues were synthesized from the Diels-Alder adduct of cyclopentadiene and maleic acid anhydride[41] (Scheme 12). After Hoffmann[42] degradation of the product obtained by ammonolysis of *diendo*-norbornenedicarboxylic anhydride (**46**), the reaction mixture was purified on an ion-exchange column, and 2-*endo*-aminobicyclo[2.2.1]hept-5-ene-3-*endo*-carboxylic acid (**47**) was isolated and then reduced with LAH to obtain the 3-*endo*-hydroxymethylbicyclo[2.2.1]hept-5-enyl-2-*endo*-amine (**39a**). The double bond of the ester hydrochloride of the amino acid (**48**) was saturated with hydrogen in the presence of PtO_2 as catalyst, and the base **50** that was released from the ester salt **49** by treatment with Et_3N was reduced with LAH to the aminoalcohol **37a** (Scheme 12).

The *N*-methyl (**36b-39b**) and *N*-benzyl derivatives (**36c-39c**) of the aminoalcohols (**36a-39a**) were made from the amino acids[43, 44] (Scheme 13). The carbamates **56-59** prepared from the hydrochlorides of the amino acid esters **48**, **49**, **51** and **52** with ethyl chloroformate were reduced with LAH to give 2-*exo*-methylaminobicyclo[2.2.1]heptane-3-*exo*-methanol (**36b**), 2-*endo*-methylaminobicyclo[2.2.1]heptane-3-*endo*-methanol (**37b**), 2-*exo*-methylaminobicyclo[2.2.1]hept-5-ene-3-*exo*-methanol (**38b**) and 2-*endo*-methylamino-bicyclo[2.2.1]-



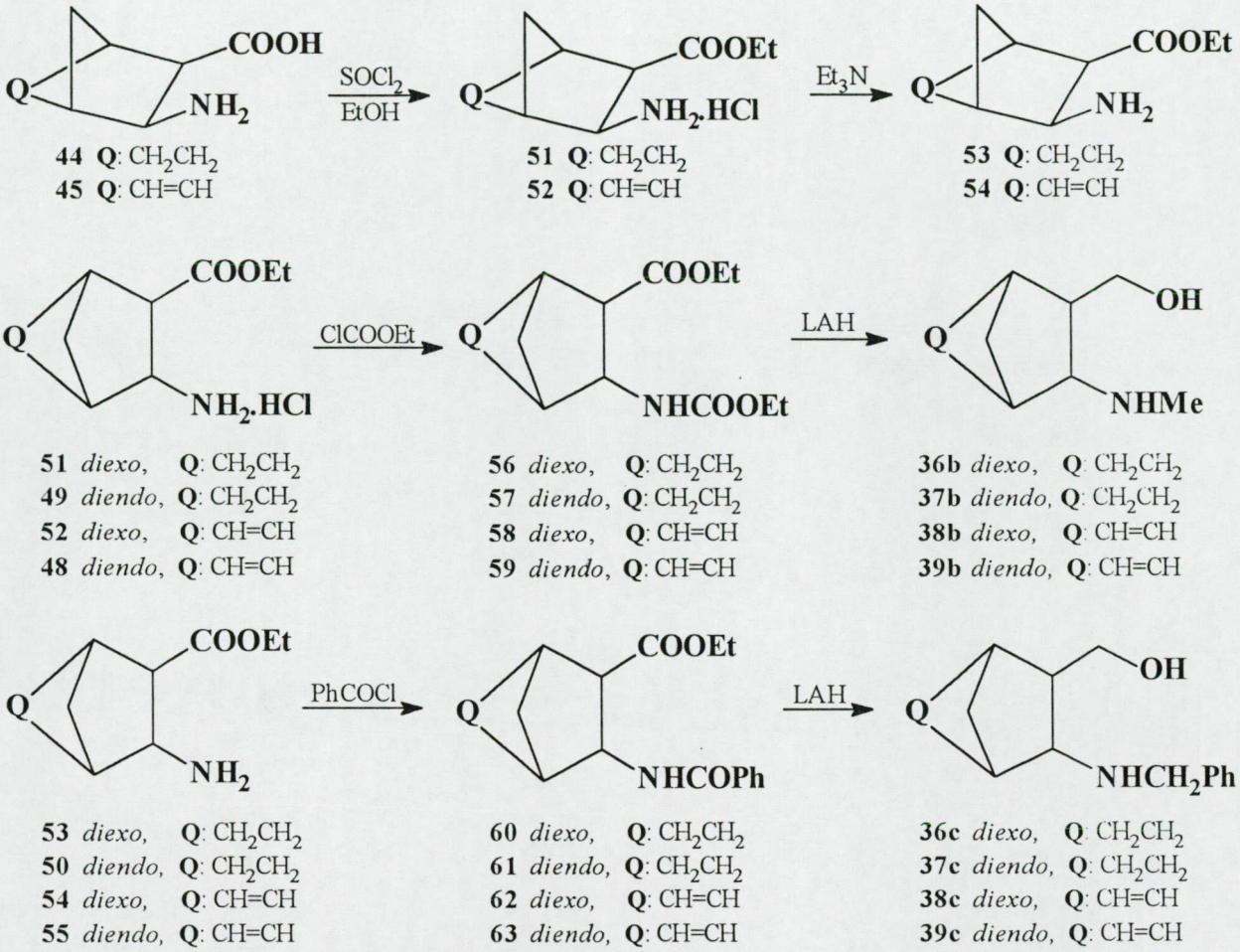
Scheme 12

hept-5-ene-3-*endo*-methanol (**39b**). With benzoyl chloride, the benzamides **60-63** were prepared; on subsequent reduction with LAH, these gave the *N*-benzyl-substituted amino-alcohols (**36c-39c**).

The unsubstituted aminoalcohols and the methyl and benzyl derivatives (**36-39**) were converted with phenyl isothiocyanates into thioureas (**64-67**) in nearly quantitative yields (Scheme 14). On refluxing in EtOH containing 20% HCl,[45, 46] the thioureas were cyclized to the saturated and 6,7-unsaturated *diendo*- and *diexo*-2-phenylimino-5,8-methano-3,1-benzo-thiazines (**68a-c–71a-c**).

However, the acid-catalysed cyclization can follow two reaction paths, since the nucleophilic attack of the sulphur on the non-cyclic methylene carbon results in the formation of 1,3-thiazines (**68-71**), whereas the attack of the oxygen of the hydroxythiourea intermediates **64-67** on the thiocarbonyl carbon leads to the formation of tricyclic 1,3-oxazine-2-thiones.

Two of the latter compounds (**72b** and **73b**) could be isolated besides **68b** and **69b**; these 1,3-oxazine-2-thiones were already prepared earlier.[43, 46]



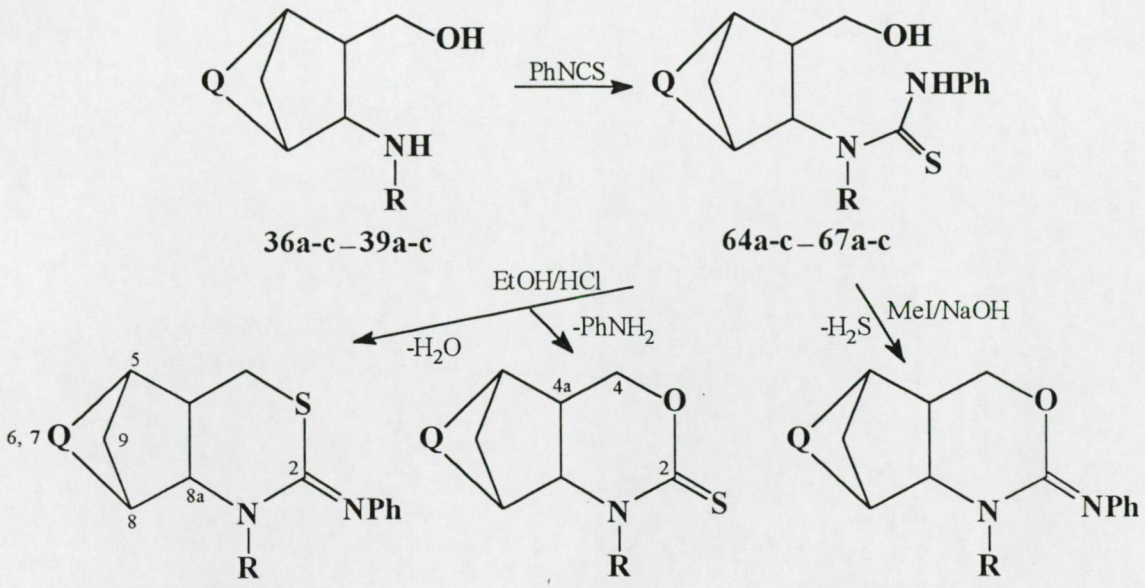
Scheme 13

Monocyclic 1,3-oxazinethione analogues were prepared in 87% yield by Guseva *et al.*[47] by refluxing aliphatic hydroxythioureas in toluene. They assumed amine elimination and the formation of an isothiocyanate intermediate.

From the reactions of the thioureas (**64-67**) with methyl iodide in the presence of a base, isothiuronium salts were prepared, which were cyclized to 1,3-oxazines (**74a-c–77a-c**).[48] In the case of **77b**, the isothiuronium salt intermediate was also isolated.

The thioureas **78a-c** prepared from the isomeric aminoalcohol **1** by treatment with isothiocyanates were cyclized with HCl to the 1,3-oxazines **79a-c** (Scheme 15). The acid-catalysed cyclization gave heterogeneous products. The main oxazine compounds (**79a-c**)

were isolated; in contrast with the isomers **68-71**, however, the analogous thiazines could not be prepared.



36a-c – 39a-c **64a-c – 67a-c**

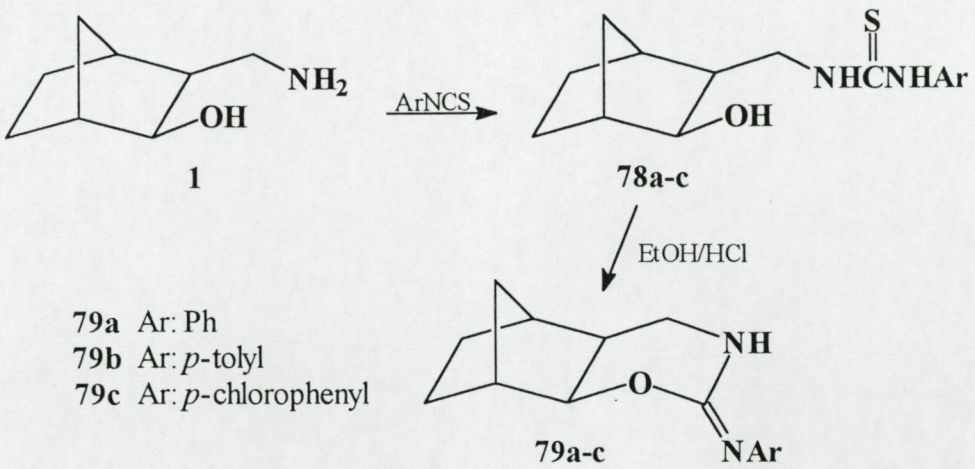
68a-c – 71a-c **72b, 73b** **74a-c – 77a-c**

Compound		Isomer	R	Q
36a, 64a, 68a,	**74a**	*diexo*	H	CH_2CH_2
37a, 65a, 69a,	**75a**	*diendo*	H	CH_2CH_2
38a, 66a, 70a,	**76a**	*diexo*	H	CH=CH
39a, 67a, 71a,	**77a**	*diendo*	H	CH=CH
36b, 64b, 68b, 72b,	**74b**	*diexo*	CH_3	CH_2CH_2
37b, 65b, 69b, 73b,	**75b**	*diendo*	CH_3	CH_2CH_2
38b, 66b, 70b,	**76b**	*diexo*	CH_3	CH=CH
39b, 67b, 71b,	**77b**	*diendo*	CH_3	CH=CH
36c, 64c, 68c,	**74c**	*diexo*	*p*-tolyl	CH_2CH_2
37c, 65c, 69c,	**75c**	*diendo*	*p*-tolyl	CH_2CH_2
38c, 66c, 70c,	**76c**	*diexo*	*p*-tolyl	CH=CH
39c, 67c, 71c,	**77c**	*diendo*	*p*-tolyl	CH=CH

Scheme 14

The amino-imino tautomerism of monocyclic 2-imino-substituted 1,3-oxazoles, 1,3-oxazines, 1,3-thiazoles and 1,3-thiazines has already been studied in detail.[49] Depending on the phase (solid or solution) investigated, the various methods of examination (IR, ^{1}H and ^{13}C NMR and X-ray diffraction) indicated that in some cases the amine, and in other cases the

imine form predominated.[50-56] For thiazoles and thiazine derivatives, the exact ratio of the tautomers was recently determined by ^{15}N NMR spectroscopy.[57]



1 **78a-c** **79a-c**

79a Ar: Ph
79b Ar: *p*-tolyl
79c Ar: *p*-chlorophenyl

Scheme 15

The norbornenes and norbornanes can easily be recognized from the NMR signals of H-6,7 and C-6,7 in the ^{1}H and ^{13}C NMR spectra, respectively. The two double doublets of the olefinic protons in the unsaturated compounds, each of 1H intensity, appear between 5.90 and 6.30 ppm [merged into a singlet of 2H intensity in the cases of **71a** and **71b**], whereas the signals of the methylene protons in the saturated analogues, overlapping those of the group at position 9, appear in the region 0.9-1.9 ppm. For the norbornenes, the C-6,7 lines lie between 134.6 and 140.6 ppm, whereas the signals of the saturated carbon atoms of the norbornane compounds appear at 20-30 ppm.

The thiazines **68-71** and oxazines **72-77** can be identified through the chemical shifts of H-4 and C-4. In the ^{1}H NMR spectra of the former compounds, the signals of the non-equivalent methylene protons appear at 2.4-2.95 ppm, whereas for the latter compounds they appear in the region 3.65-4.30 ppm, as anticipated.[59] The regions of the corresponding carbon chemical shifts for the two types of compounds, again as expected,[59] are at 26.7-31.1 and 65.5-69.8 ppm, respectively.

A considerable difference is found as concern the chemical shift of C-2. In the thiazines, the adjacent sulphur atom causes a marked downfield shift of the C-2 signal as compared with that in the oxazines. The C-2 signal for the thiazines appears at 156.7-160.0 ppm, whereas for the oxazines (**74-77**) it lies at 144.5-148.6 ppm. The chemical shift of C-1'

(2-phenyl group), however, reveals an opposite effect, though to a lesser extent: it is found in the interval 147.1-151.6 ppm for the thiazines and 151.5-1 53.4 ppm for the oxazines.

The *diendo* or *diexo* annelation of the hetero- and carbobicyclic rings is indicated by the multiplicity of the H-8a signal in the region 3.0-4.09 ppm. For the *diexo* compounds, this signal is a doublet (J = 7-10 Hz) corresponding to the H-4a,8a coupling, whereas in the spectra of the *diendo* compounds it appears as a double doublet, besides the splitting originating from the H-4a,8a spin-spin interaction (the magnitude of which varies in the range 6.5-12.5 Hz in this series); another significant splitting is observed due to the H-8,8a coupling (3-4 Hz). The dihedral angle H-8–C-8–C-8a–H-8a is ~50°, whereas that in the *diexo* compounds is ~90°, and therefore no splitting can be anticipated[37] [J(H-8,H-8a) < 1 Hz].[23]

The H-8a chemical shift depends mainly on the annelation: it is 3.01-3.36 ppm for the *endo* H-8a of the *diexo* series and 3.30-3.95 ppm for the *exo* H-8a of the *diendo* compounds, in accord with the literature data on norbornane and norbornene. Values of 1.18 and 1.46 ppm have been measured for the protons of the former, and 0.96 and 1.59 ppm for those of the latter in the *endo* and *exo* configurations, respectively.[60-62]

This holds for the H-4a signal too: for **70** and **76**, the shielding is stronger (1.98-2.23 ppm) than for the isomers **71** and **77** (2.5-2.8 ppm); similarly, it is stronger for **68** and **74** (1.98-2.2 ppm) than for **69** and **75** (2.3-2.4 ppm). Again as expected, we found that the position of the H-8a signal is also slightly dependent on the saturation of the carbocycle. H-8a in the norbornenes is more shielded in the *diexo* isomers, with chemical shifts of 3.01-3.30 ppm, while for the norbornanes shifts between 3.10 and 3.36 ppm were observed. For the *diendo* compounds, the chemical shift interval is 3.3-3.6 ppm for norbornanes, whereas for the norbornene analogues it is 3.61-3.95 ppm.

There are significant differences in the H-5,8 chemical shifts of the saturated and unsaturated compounds. In the latter group of compounds, the $-I$ effect of the olefinic group causes a downfield shift: the H-5 signal is in the region 2.5-2.9 ppm, while that of H-8 lies at 2.54-3.25 ppm. For the norbornanes, however, the two shift intervals are 2.05-2.25 and 2.05-2.7 ppm, respectively.

In the ^{13}C NMR spectra of the norbornenes, the C-5,8, signals display a downfield shift, due to the greater α-effect[59] of the olefinic bond (45.2-8.3 and 46.2-9.5 ppm, respec-

tively, as compared with the observed values of 39.5-2.1 and 39.6-44.1 ppm, respectively, for the norbornanes.

In the spectra of the norbornanes, the C-4a and C-8a lines are shifted upfield in consequence of the steric hindrance between H-6,7 (*endo*) and the NR moiety or H-4 (steric compression shift: carbons bearing sterically hindered groups are more shielded[63]).

For compounds **69** and **75**, the mean shift of the C-4a signal is 42.2 and 38.3 ppm, respectively; this means a field effect of 5.2 ppm as compared with those in the analogues **68** and **74** (47.4 and 43.5 ppm, respectively). For the C-8a signal, the mean field effect is 4.1 and 3.9 ppm. For the norbornenes, the greater distance of the olefinic protons from H-4 and the NR group, results in the disappearance of the field effect; furthermore, for the H-4a signal, an opposite, albeit lower (average of 1.9 and 1.3 ppm) shift is observed. Steric hindrance is also present in the *diexo* compounds, but instead of H-6,7 (*endo*), H-9' (*endo*) is involved with 4-CH_2 or the NR group. This is indicated by the large field effect on the C-9 signals, which can be observed for both the norbornanes and norbornenes. The average field effects for the pairs **70-71**, **68-69**, **76-77** and **74-75** are 5.1, 4.4, 4.5 and 4.2 ppm, respectively.

It is noteworthy that *endo*↔*exo* C=N bond tautomeric equilibrium is possible in the *N*-unsubstituted compounds (**68a-71a** and **74a-77a**).. The synthesis route means that the corresponding *N*-substituted series **b** and **c** are exclusively isomers substituted on the ring nitrogen, containing an exocyclic C=N bond. The fact that neither the chemical shifts of the aromatic protons of the 2-phenylimino group, nor those of the carbon atoms in this ring (with the exception of C-1'), are altered significantly as compared with those in the *N*-substituted analogues is unambiguous evidence of the predominance of the tautomeric form containing an exocyclic C=N bond *(i.e.* an NH group in the ring) in $CDCl_3$ solution. Since this is also valid for compound **76a**, a change in the tautomeric equilibrium can not be detected in DMSO-d_6 solution either.

3. Synthesis and NMR study of norbornane- and norbornene-fused tetracyclic azetidinones[3]

In view of the great medicinal importance of the penicillins and cephalosporins containing an azetidinone ring, increasing interest is attached to β-lactams.[64] The methylene-bridged, partly saturated 1,3- and 3,1-benzoxazines, prepared earlier in our laboratory,[21a, 21b, 23] readily undergo cycloaddition reactions with chloroacetyl chloride to give fused-skeleton

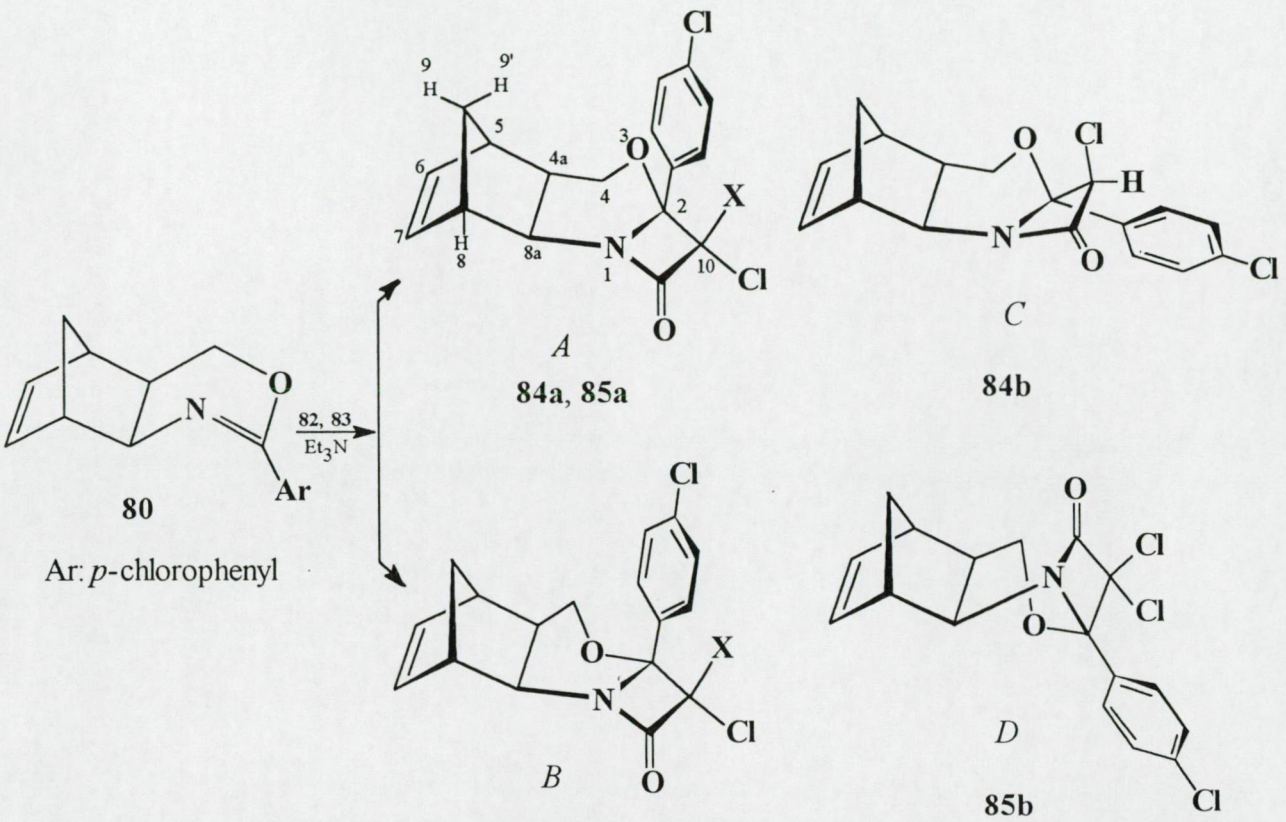
azetidinones. This extension of rigid-skeleton norbornane-fused isomeric 1,3-oxazines is of stereochemical interest. These tetracyclic β-lactams also seem promising from the point of view of biological activity, since the norbornane moiety is a component of many active compounds.[26]

The subject of the present section is the conversion into tetracyclic azetidinones of the tricyclic fused-skeleton 1,3-oxazine derivatives that we had prepared earlier from the *diexo*-3-aminobicyclo[2.2.l]hept-5-ene-2-methanol with imidates[21a, 21b, 23] or by 1,4-cycloaddition from norbornene, norbornadiene and *N*-hydroxymethylbenzamides (**32a,b** and **34b**), and also the *diendo*- and *diexo*-condensed 3,1-benzoxazines (**80** and **81**) from aminoalcohols **38a** and **39a**.[65]

The 1,3-dihydrooxazine compound formed with *diexo*-norbornene (**80**) reacts by [2+2]-cycloaddition with ketenes prepared *in situ* from chloroacetyl chloride (**82**) or dichloroacetyl chloride (**83**) with triethylamine to give a product containing a mixture of two isomers (**84a,b** and **85a,b**). An attempt was made to separate these compounds by column chromatography. Unfortunately, only the main products (**84a** and **85a**) could be obtained in pure form, but their different solubility and chromatographic behaviour permitted isolation of the epimeric mixture, in which the minor products (**84b** and **85b**) were enhanced.

Thus, the structures could be established by abstracting the spectra of the main components from the ^{1}H NMR spectrum of the mixture by means of a computer compensation method. The results strongly suggested that the structures of the main components **84a** and **85a** were *A*, while those of the minor ones, **84b** and **85b**, were *C* and *D*, respectively (Scheme 16).

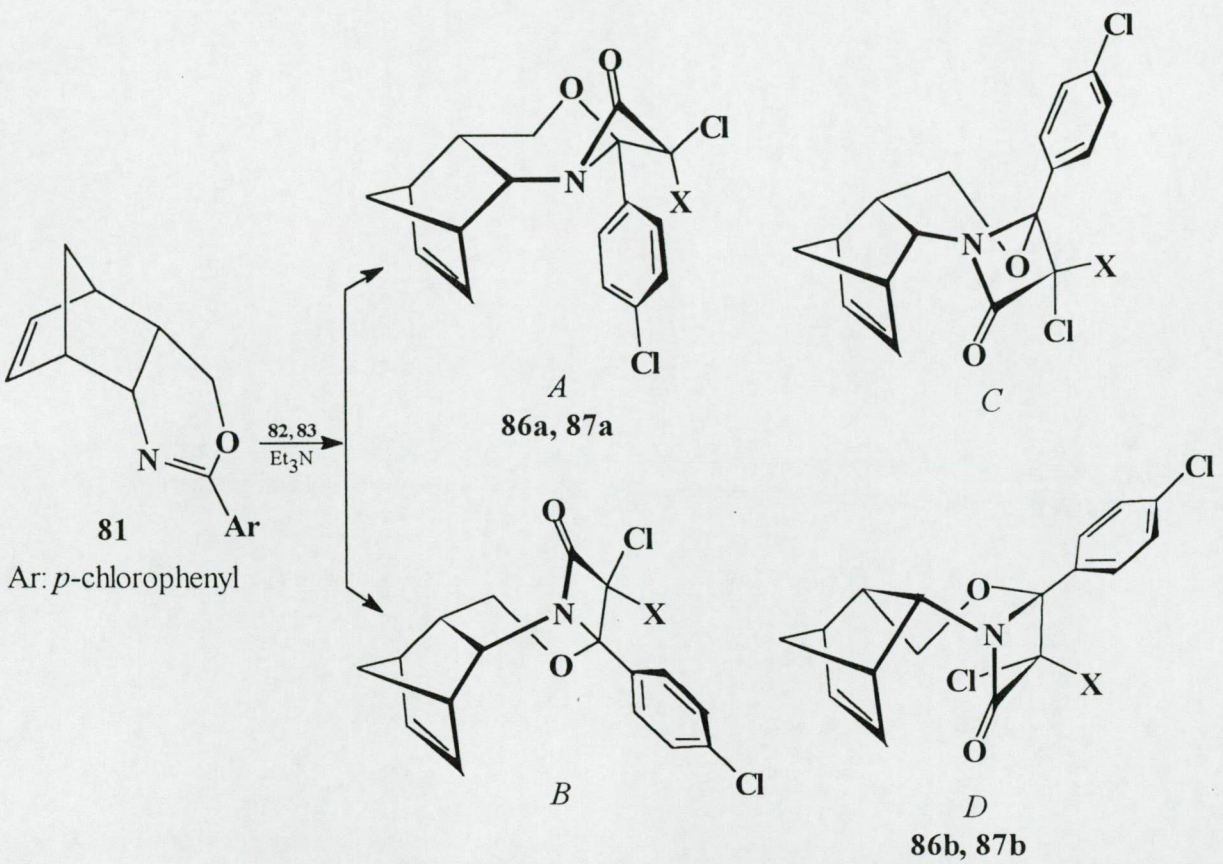
Two diastereomers each of **86** and **87** (**86a,b** and **87a,b**) were also isolated from the reaction mixture of the *diendo* derivatives; their structure elucidation has already been reported.[23] On heating to 180 °C, **87a** (mp 137-139 °C) is converted into the epimer **87b** (mp 194-195 °C), *i.e.* the configuration of the 5*S* carbon atom changes to 5*R*. Hence, the isomer **87b** is the more stable. It was found that the structures of the main comformers **86a** and **87a** are *A*, while those of the minor ones, **86b** and **87b,** are *D* (Sceme 17).



82: $ClCH_2COCl$; **83**: $Cl_2CHCOCl$; **84a,b**: X = H; **85a,b**: X = Cl

Scheme 16

Only one diastereomer each could be isolated from the mixtures of isomers **88-91** starting from the 1,3-benzoxazines **32a,b**, and of **92** and **93** from **34b** (Scheme 18). The reaction of **32a** with chloroacetyl chloride takes place in two directions: besides the azetidinone **88**, a 1,3-oxazino[2,3*b*]-1,3-oxazine-4-one derivative **94** invilving fusion to norbornane and containing a nitrogen bridgehead could also be isolated, *i.e.* a derivative formed by reaction with two equivalents of chloroacetyl chloride. The synthesis of monocyclic dihydrooxazines from acyclic imines by reaction with acetyl chloride has been reported.[66] The formation of diketene is assumed as the explanation of the reaction. Relatively simple bicyclic compounds of the aza orthoester-type have been prepared from dihydro-1,3-oxazine by means of epoxide addition.[67]



82: $ClCH_2COCl$; **83**: $Cl_2CHCOCl$; **86a,b**: X = H; **87a,b**: X = Cl

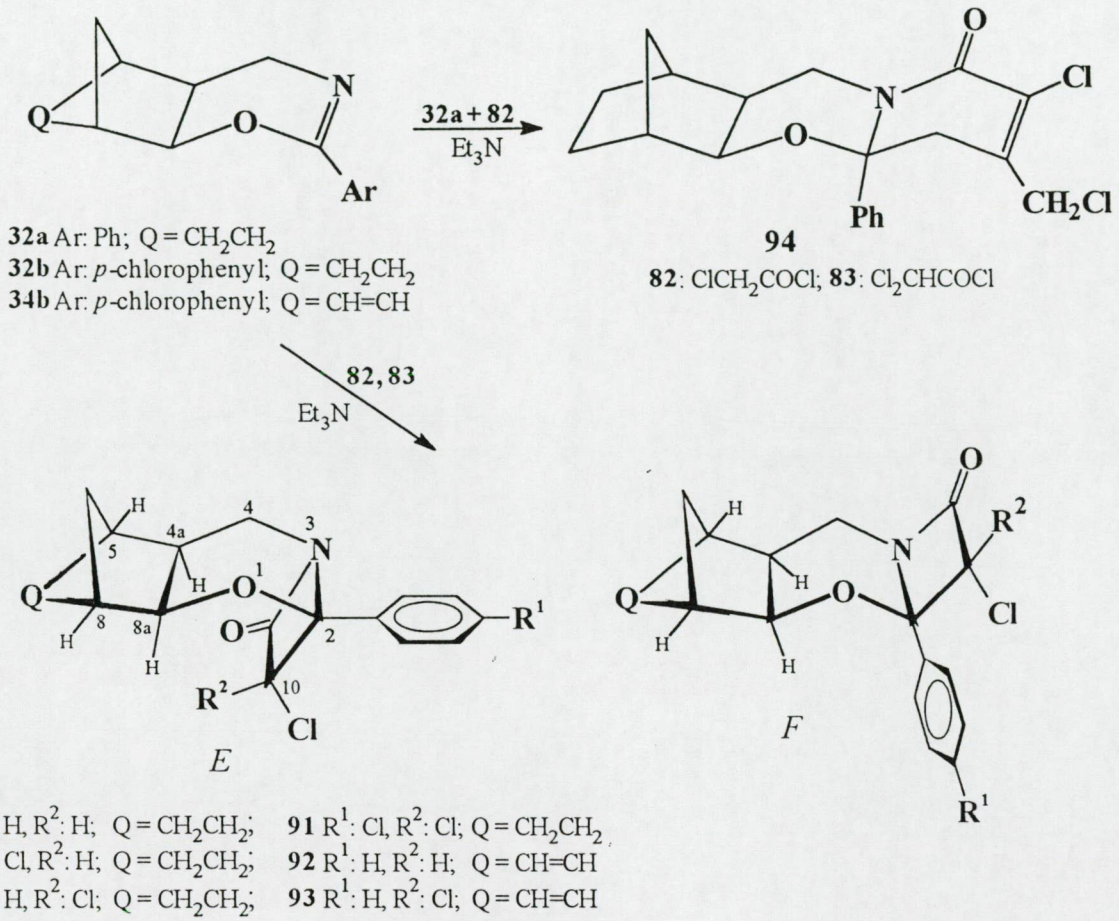
Scheme 17

NMR spectroscopic study

A comparison of the spectral data reveals that in isomer **85a**, isolated in the pure state, the configuration of C-2* is *R* (*A*), *i.e.* the phenyl substituent is in the *endo* position; the other component of the mixture, **85b**, is the 2*S* *exo*-phenyl epimer (*D*). The main facts supporting this conclusion are as follows:

a) The H-9 and H-9' signals for **85a** show a considerable upfield shift (0.62 and 0.46 ppm) as compared with those for epimer **85b**. This is due to the anisotropic effect[59] of the phenyl ring approaching H-9,9', causing shielding of the hydrogens situated "above" the plane of the ring.

* In the spectroscopic sections, the numbering is uniform for all compounds investigated, in order to facilitate comparison of the analogous data.



32a Ar: Ph; Q = CH_2CH_2
32b Ar: *p*-chlorophenyl; Q = CH_2CH_2
34b Ar: *p*-chlorophenyl; Q = CH=CH

94

82: $ClCH_2COCl$; **83**: $Cl_2CHCOCl$

E *F*

88 R^1: H, R^2: H; Q = CH_2CH_2; **91** R^1: Cl, R^2: Cl; Q = CH_2CH_2
89 R^1: Cl, R^2: H; Q = CH_2CH_2; **92** R^1: H, R^2: H; Q = CH=CH
90 R^1: H, R^2: Cl; Q = CH_2CH_2; **93** R^1: H, R^2: Cl; Q = CH=CH

Scheme 18

b) The same effect can be observed for the H-8 signal; this signal is upfield-shifted by 0.96 ppm as compared with that for **85b**.

c) A downfield shift by 0.76 ppm, as compared with **85b**, is found for the H-8a signal; this shift is due to the deshielding effect of the coplanar carbonyl group.[59]

This fact may be made the starting point in the determination of the C-10 configuration in the analogous monochloro-substituted analogue **84a**. As compared with the epimer **84b**, the 2*R* configuration (*endo* phenyl group) can be inferred from the upfield shifts of the H-8,9,9' signals and the downfield shift of the H-8a signal, analogously to the case of **85a**. On the other hand, conformation *A*, analogous to that of the dichloro compound **85a**, follows from the similar coupling constants *J*(4,4a) and *J*(4',4a), 7.8 and 8.6 Hz. In the event of a similar stereostructure, a chlorine in the *trans* position should give rise to a similar,

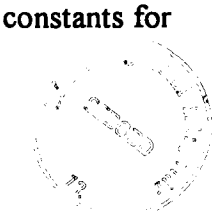
anomalous shift relationship ($dH_a > dH_e$) for the C-4 methylene hydrogens. As this is not observed, the *cis* arrangement of the phenyl and chlorine substituents relative to the azetidinone ring, and hence the *R* configuration at C-10, are apparent. The assignments of the signals of the axial and equatorial methylene hydrogens are given on the basis of the relationship $J(4',4a) > J(4,4e)$, *i.e.* starting from the Karplus relation,[37] $J(a,a) > J(a,e) > J(e,e)$. The steric position determined in this way for the chlorine atom is explained by the circumstance that, due to the coplanarity of the C_2-O_3 and C_{10}-Cl_{trans} bonds, a strong electrostatic repulsion would appear between a *trans* chlorine atom and O-3; the molecule can avoid this by assuming the *R* configuration at C-10. This phenomenon may be regarded as a special case of the anomeric effect[68, 69] that is well known in carbohydrate chemistry.

Thus, it is readily understandable that conformation *D* is the preferred one for **85b**; unambiguous evidence in support of this is provided by the significantly different, but relatively high values of the coupling constants $J(4,4a) = 6.6$ Hz and $J(4',4a) = 12.2$ Hz. The anomaly in the chemical shifts of the C-4 methylene hydrogens, observed in the case of **85a**, is absent here; the signals of H-8 and H-9,9' are shifted downfield in comparison with those for **85a**, due to the proximity of the carbonyl oxygen. On the other hand, the H-8a signal suffers an upfield shift, since H-8a is farther removed from the carbonyl group and, as it is above the plane of the phenyl ring, it is more shielded.

Whereas the spectral data indicate the analogous conformation *A* for **84a** and **85a**, the spectral parameters of **84b** suggest *C* as the preferred conformation. The most important evidence for this is given again by the similar and smaller coupling constants $J(4,4a) = 6.4$ and $J(4',4a) = 8$ Hz, and also by the upfield shifts, *i.e.* slightly smaller for the signal of H-9, but higher for that of H-8 (0.55 and 1.05 ppm), than in the case of **84a** (the corresponding differences in the pair **85a,b** are 0.62 and 0.96 ppm).

Differentiation of structures **86** and **87** is based on the changes in the chemical shifts of the olefinic protons as compared with **81**. These shifts are decreased significantly for **86a** and **87b**. This can be explained by the anisotropy of the phenyl ring near the olefinic protons (~3 Å) in structures **86a** and **87b**.[23]

As regards the conformation, the values of the 4,4a and 4',4a couplings are the deciding factor. In conformation *A* the corresponding dihedral angles are ~35° and ~165°, while in *B* they are ~40° and ~90°, respectively. As the corresponding coupling constants for



86a are 9.7 and 8.0 Hz, the preferred conformation is *A*. It should be noted that, for the same reason, conformation *C* should be taken into account for isomer **87a**. Here H-7 and C=O, H-4'(a) and the *endo* chlorine atoms are near each other, but no sign of such a situation is observed in the spectrum. The magnitudes of the 4,4a and 4',4a couplings point to the fact that the anomalous chemical shift relationship δH-4(e) < δH-4'(a) for **81** is normalized here: δH-4(e) = 4.18 ppm and δH-4'(a) = 3.32 ppm. Consequently, the H-4 signal for **86a** is shifted downfield by 0.4 ppm, and the H-4' signal by 1 ppm in the opposite direction. This significant increase in the shielding of H-4' is also caused by the anisotropic effect of the aromatic ring, giving additional proof of conformation *A*. For conformation *B*, the anisotropic effect would appear in the signal of H-7, but not those of H-6 and H-4'.

In the spectra of the two isolated dichloro compounds, the H-8a signal appears unchanged as a double doublet, as in the case of **81** and **86a**. Consequently, the *diendo* annelation of the norbornene skeleton and the hetero ring is unaltered, as predictable. Thus, only structures **86b** and **87b**, differing in the annelation of the azetidinone ring, need be taken into consideration. From analogous spectral data for the monochloro derivative **86a**, one isomer has an **86b**-type structure and an *A*-type conformation, while the other one has a structure **87b**, similar to **87a**, and conformation *D*. This is verified by the following facts:

a) The shielding of H-6,7 in **87b** is not increased, but decreased as compared with that for **81**. The 0.4 ppm downfield shift of the H-7 signals is caused by the proximity of the carbonyl oxygen.

b) Conformation *D* is proved by the values of the 4',4 and 4,4a couplings (9.3 and 6.2 Hz for **87b** and 7.3 and 7.2 Hz for **86b**).

c) H-8a in **87b** is shielded by 0.7 and 0.6 ppm as compared with **86b** and **86a** having conformation *D*, where the anisotropy of the carbonyl group (which is much closer to H-8a) causes a downfield shift of the H-8a signal. Owing to the mutual positions of H-8a and the carbonyl group in conformation *D* of structure **87b**, this effect is manifested as an opposite shift.

d) The 4,4'-methylene hydrogens in **87b** are shielded by the anisotropy of the four-membered ring and that of the C–Cl bond. In the case of H-4', this does not compensate for the strong shielding effect of the phenyl ring that is operative in **86b**. Consequently, H-4 is more shielded, while H-4' is deshielded as compared with **86b**, but the H-4' signal for **87b** is

more shielded than that for **81**. Thus, in **87b**, the normal relationship δH-4(e) > δH-4'(a) is again observed, but the difference ΔδH-4,4' is decreased.

Structures of linearly fused azetidinones 88-94

In view of the tabulated data[3], structure *E*, *i.e.* the *S* configuration of C-2, can be assumed on the basis of the following arguments:

a) H-4a is less shielded than in **84a,b** and **85a,b** (the H-4a signal is shifted from 1.71-1.98 ppm into the region 2.30-2.45 ppm).

b) The H-8a shifts cannot be compared directly, as the neighbouring nitrogen is replaced by oxygen. However, it is known[59] that the presence and vicinity of an amide nitrogen, *e.g.* in cyclohexanes, causes a geminal proton deshielding that is about 0.7 ppm greater than in the case of an ether oxygen substituent; accordingly, the observed downfield shift of the H-8a signal can be rationalized only by structure *E*.

c) A deciding argument in favour of configuration *E* is the very marked shift difference in the H-8a signals of the mono- and dichloro derivatives (0.36, 0.36 and 0.35 ppm for the pairs **88-90**, **89-91** and **92-93**, respectively). The downfield shifts observed for the dichloro compounds are explained by the anisotropic effect of the chlorine atom.[59] In the *F* isomers, both chlorine atoms are distant from H-8a, whereas in epimer *E* the chlorine *trans* to the phenyl ring is very close to H-8a. This is evidence of the *cis* arrangement of the chlorine and phenyl substituents on the azetidinone ring, and of the *S* configuration at C-10 in the monochloro compounds (**88**, **89** and **92**).

The IR spectrum of **94** does not display the characteristic azetidinone carbonyl band.[70] Instead, the amide-I band at 1672 cm^{-1}, typical of simple amides, is observed, precluding the possibility of a β-lactam structure. The amide-III band, characteris-tic of *N*-substituted δ-lactams,[58] is found at 1415 cm^{-1}. Besides the characteristic bands of the aromatic and aliphatic groups, the spectrum exhibits intense bands between 1300 and 945 cm^{-1}, due to polar bonds such as C-O, C-Cl or C-N.

The ^1H NMR data afford evidence of the presence of the norbornane skeleton and the phenyl ring. The downfield shift of the H-4,4' signals can be accounted for by the anisotropy of the carbonyl group. The doublet split of the H-8a signal proves the unchanged *diexo* annelation of the norbornane and oxazine rings; the considerable upfield shift (~0.8 ppm) of

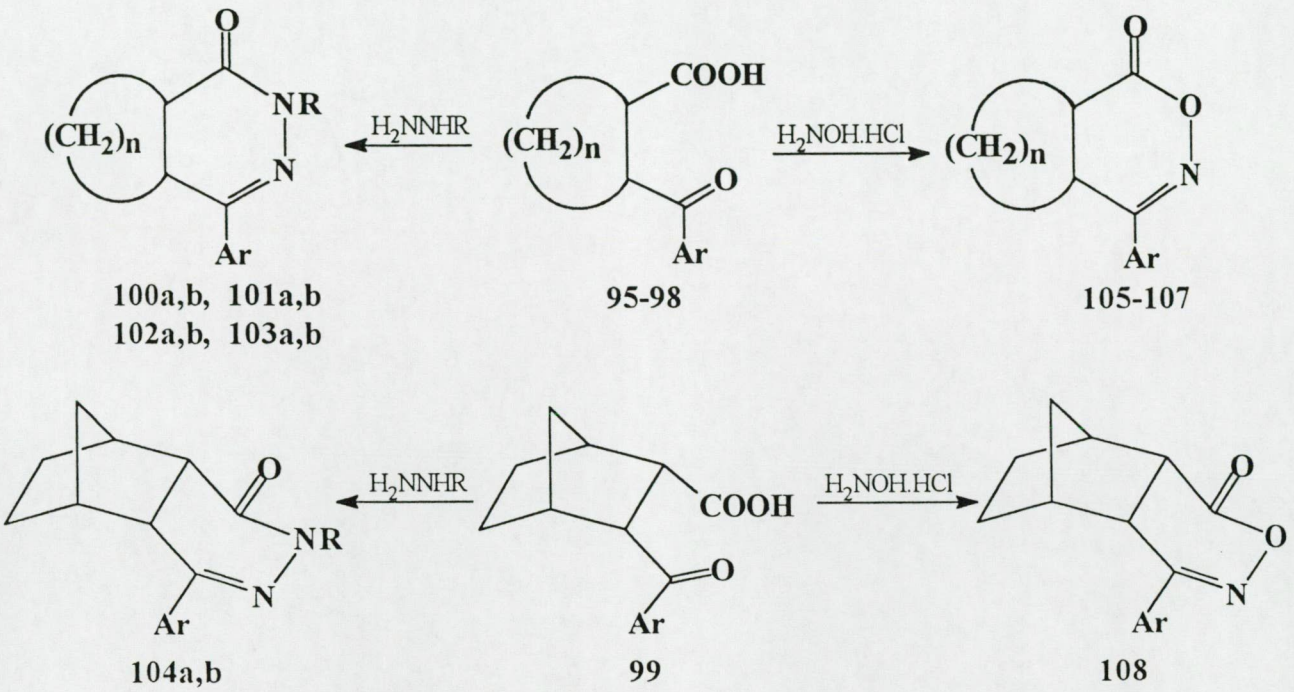
this doublet relative to that for the azetidinones **88-93**, together with the somewhat smaller (~0.6 ppm) H-4a shift in the same direction, reflects the parallel, "*endo*" position of the phenyl ring (*R* configuration at C-2), since the anisotropic effect of the phenyl ring increases the shielding of H-4a,8a. In accord with this, H-9' (*endo*) is less shielded than in **88-93**. The conformation of the oxazine ring is the same as in the azetidinones **88-93**; this follows unequivocally from the coupling constants $J(4,4a) = 8 < J(4',4a) = 11.5$ Hz. In this conformation, if the configuration were C-2*R* (*exo* phenyl), increased shielding of H-9' would be observed, as a consequence of the proximity of the phenyl ring. The downfield shift of the H-5 signal by about 0.2 ppm is explained by the vicinity of the carbonyl group. Finally, the increased shielding of H-7 (*endo*) is also attributable to the anisotropy of the phenyl group.

4. Preparation and steric structure of 3(2*H*)-pyridazinones, 1,2-oxazine-6-ones and anthracene derivatives[4]

This section describes the synthesis of several small-ring (cyclopropane- and cyclobutane-fused) analogues of some of the earlier heterocycles. An important feature of the compounds described here is that the synthons used are 2-aroyl-1-cycloalkanecarboxylic acids[71-73] which yield fused-skeleton heterocycles containing an aroyl group in the neighbourhood or on a bridgehead carbon atom in the fused heterocycle. The 2-aroylcyclohexanecarboxylic acids and their cyclic derivatives have been reported to exert anorectic and hypotensive effects.[74, 75]

cis-2-*p*-Toluoylcyclopropane- (**95**) and -cyclobutanecarboxylic acids (**96**), *cis*- (**97**) and *trans*-2-*p*-toluoylcyclohexanecarboxylic acids (**98**) and *diendo*-3-*p*-toluoylbicyclo-[2.2.1]heptane-2-carboxylic acid (**99**), prepared according to the literature[71, 73] or analogously from *diendo*-norbornane-2,3-dicarboxylic anhydride and toluene by the Friedel-Crafts reaction, were cyclized with hydrazine hydrate and phenylhydrazine to the cyclopropane-, cyclobutane- and cyclohexane-*cis*-fused 4,5-dihydro-3(2*H*)-pyridazinones (**100a**, **101a** and **102a**), the corresponding *trans* derivative (**103a**) and their 2-phenyl derivatives (**100b-103b**) (Scheme 19).

For pharmacological aims, the analogous norbornane-*diendo*-condensed pyridazin-3(2*H*)-ones (**1104a,b**) were also prepared. As potential drugs, the non-condensed or differently substituted derivatives of the 4,5-dihydropyridazinones have already been synthesized.[73, 75, 76]



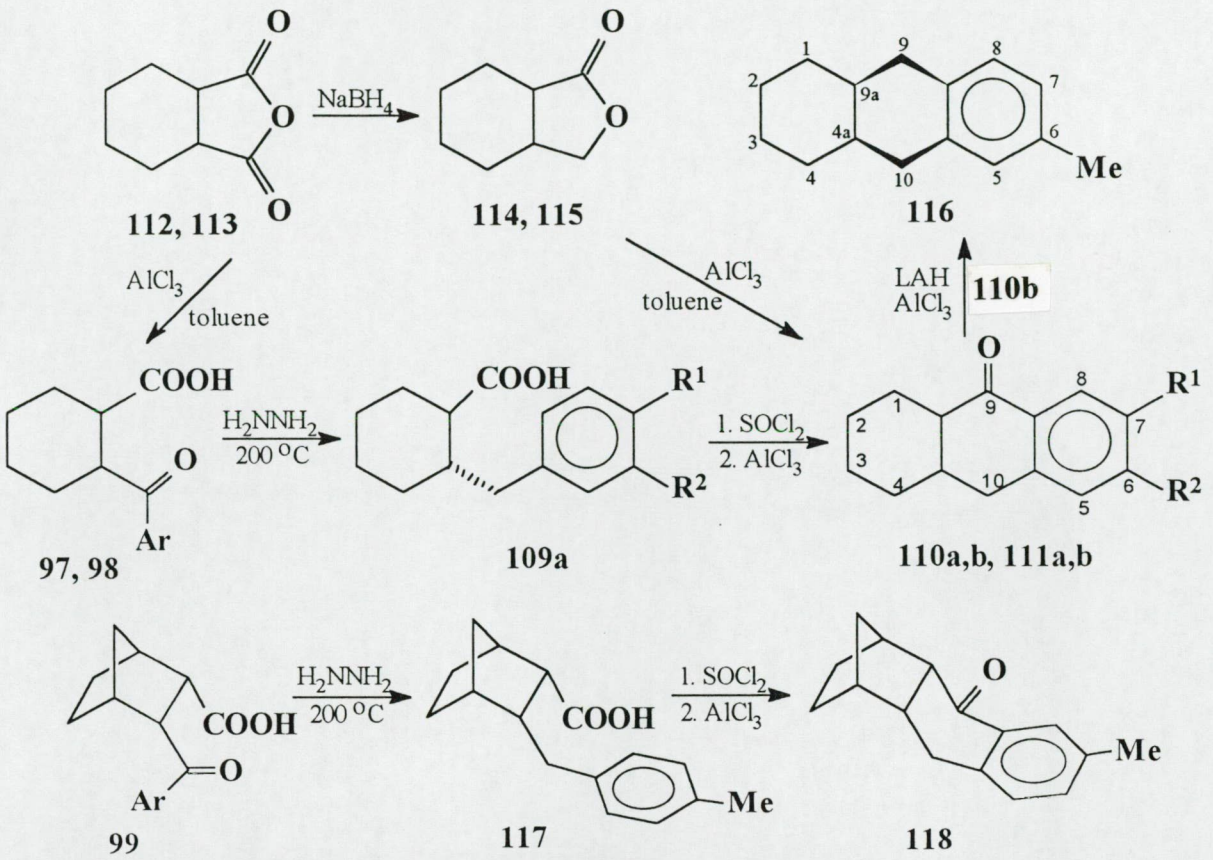
cis: **95-97, 100-102, 105, 106**; *trans*: **98, 103, 107**; **a**: R = H; **b**: R = Ph; Ar = *p*-tolyl
n = 1 (**95, 100, 105**); n = 2 (**96, 101**); n = 4 (**97, 98, 102, 106, 107**)

Scheme 19

With hydroxylamine hydrochloride, synthons **95** and **97-99** yielded the cyclopropane-*cis*- (**105**), cyclohexane-*cis*- (**106**) and *trans*- (**107**), and norbornane-*diendo*-condensed (**108**) 4,5-dihydro-1,2-6*H*-oxazine-6-ones.

As the aroylcyclohexanecarboxylic acids also appeared to be suitable for the synthesis of partially saturated anthracene derivatives, the synthons **97-99** were reduced by the Wolff-Kishner method. The product obtained from both **97** and **98** was the *trans*-2-*p*-tolylmethyl-cyclohexanecarboxylic acid[77-79] (**109a**), while the reduction of **99** led to the corresponding norbornane derivative **117** (Scheme 20).

The isomerization of the *cis* compound **97** presumably took place during the vigorous hydrazine reduction (200 °C, KOH). For dihydrouracils, *cis-trans* inversion on the action of acids, bases or heating is known.[80] In the present case, the isomerization **97**→**109a** was proved by further reactions. Reduction and cyclization of the *cis* **97** and the *trans* synthon **98** gave the same *trans* compound **111a**. The configuration of the fixed methylene-bridged *diendo* analogue **99** was not changed by the reduction.



cis: **97, 110, 112, 114**; *trans*: **98, 109, 111, 113, 115**;
Ar = *p*-tolyl; **a**: R^1 = Me, R^2 = H; **b**: R^1 = H, R^2 = Me

Scheme 20

From *cis*-4-cyclohexene-1,2- and *diendo*-5-norbornene-2,3-dicarboxylic anhydride, the preparation of the oxocarboxylic acids of types **97-99** under similar conditions was unsuccessful because of the saturation of the double bond in the Friedel-Crafts reaction. Thus, for the synthesis of the *cis*-hexahydroanthrone, the *cis*-hexahydrophthalic anhydride **112** was reduced with $NaBH_4$ to the *cis*-lactone **114**, which was then reacted with toluene in the presence of a large excess of $AlCl_3$. This reaction directly gave *cis*-6-methyl-1,2,3,4,4a,9a-hexahydroanthrone (**110b**) instead of the 7-methyl derivative (**110a**).

Starting from *trans*-hexahydrophthalic anhydride (**113**), *trans*-6-methylanthrone **111b** was formed in the same way. Thus, acylation, Wolff-Kishner reduction and cyclization of the *trans* anhydride **113** yields the *trans*-7-methylhexahydroanthrone (**111a**), whereas the route starting with reduction of **113**, followed by simultaneous Friedel-Crafts acylation and cyclization, furnishes the regioisomeric 6-methylhexahydroanthrone **111b**.

On the cyclization of **117** in the presence of $AlCl_3$, the tetracyclic 1,4-methylene-bridged hexahydroanthrone **118** containing the norbornane *diendo*-fused structural moiety was formed. Reduction of 6-methyl-*cis*-hexahydroanthrone (**110b**) with LAH/$AlCl_3$ gave the corresponding *cis*-octahydroanthracene **116**. The syntheses of *cis*- (**110a**) and *trans*-octa-hydroanthracen-9-one (**111a**) were described earlier[77-79] and the preparation of 6-methyl-1,2,3,4,4a,9,9a,10-octahydroanthracene (**116**) by reduction of **110a** is also known.[77] Mathur and Bhargava applied the Wolff-Kishner method to reduce **110**, obtained from **97** by reduction with hydrazine and subsequent cyclization, and reported the isolation of crystals of **116** with mp 126 °C in very good yield (90.3%).[77]

We repeated their work with **110b**, but the reduction and cyclization gave a colourless liquid (yield 64%). ^{1}H NMR indicated that the product contained some starting **110b** (11%) besides **116**. Reduction of **110b** with LAH proved to be more convenient; for isolation, a HPLC method was applied, which resulted in **116** (35% of the distilled product), mp 77-80 °C.

For **110b**, the *cis* annelation of the cyclohexane and cyclohexenone rings is proved by the broad ^{1}H NMR signal (~15 Hz) of the annelated methine hydrogen vicinal to the carbonyl group. It also shows that the carbonyl group is axial and that the methylene group in the cyclohexenone ring is equatorial, which is in accordance with the higher spatial requirements of the methylene group as compared with those of the carbonyl group.[81] The corresponding signal width of the *trans* analogues **111a,b** is ~30 Hz. For **111a,b**, this signal appears 0.5 ppm upfield of that measured for **110b**, which is a consequence of the equatorial→ axial change.[13,59] Similarly, the 15 Hz signal width and the downfield shift of the signal of the hydrogen vicinal to the carbonyl group as compared with the signals for the *trans* isomers **103a,b** suggest a conformer containing an axial carbonyl group for the *cis*-annelated compounds **102a,b**.

This also holds for the former of the *cis-trans* pair **106-107**: the signal of the methine hydrogen vicinal to the carbonyl group is sharper (signal width ~8 Hz; ~30 Hz for **106**) and downfield shifted (by 0.63 ppm). Thus, the carbonyl group is axial to the cyclohexane chair ring in the preferred conformation.

Further proof of the *cis-trans* structures is that the sum of the cyclohexane carbon

shifts is 8.7 ppm smaller for *cis* **110b** than for *trans* **111b**.[59] For **111a** and **111b**, the *trans*-annelated structure follows from the same magnitude of the carbon shifts.

For **100a,b** and **105**, the significantly different shieldings and signal splittings of the two cyclopropane methylene hydrogens are noteworthy. The anisotropic effect of the hetero ring causes a strong upfield shift of the signal of the methylene hydrogen which is situated above the ring and *trans* to the vicinal hydrogens. The assignment based on the higher *cis* vicinal coupling[59] is beyond any doubt. For **104a,b** and **108**, the double doublet pattern of the signal of the heterocyclic annelated hydrogens[23] unambiguously proves the *diendo* annelation of the hetero ring and the norbornane moiety.

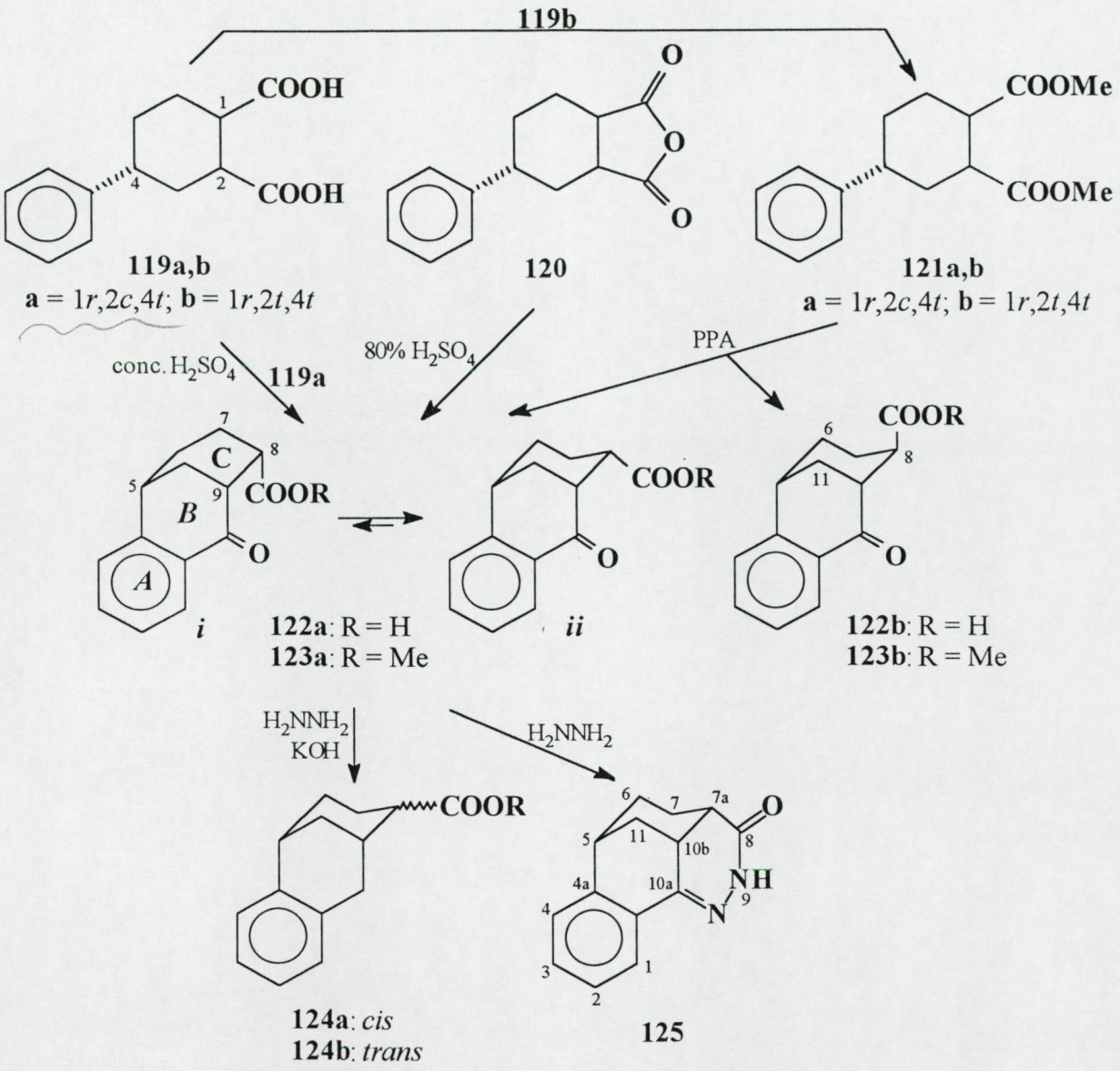
For **111b**, the 6 position of the methyl group was proved by NOE measurements.

5. Synthesis and structure of methanobenzocyclooctene derivatives[5]

As starting compound, *cis*-4-cyclohexene-1,2-dicarboxylic anhydride was applied;[82] through the addition of benzene to the double bond, this furnished 4*t*-phenylcyclohexane-1*r*,2*c*-dicarboxylic acid (**119a**) with a phenyl group equatorial[83] to the neighbouring carboxy group. It was thought that the 4-phenyl substituent on cyclohexane-1,2-dicarboxylic acid might provide a good opportunity to construct highly condensed systems by intramolecular acylation of the phenyl substituent with the 2-carboxy group. These systems containing two functional groups are suitable for the preparation of heterocycles and they provide good starting molecules for the production of new pharmacologically active derivatives as target compounds.

When heated in concentrated H_2SO_4, *trans*-4-phenylcyclohexane-*cis*-1,2-dicarboxylic acid **119a** or in 80% H_2SO_4, the anhydride **120** yielded 10-oxo-5,6,7,8,9,10-hexahydro-5,9-methanobenzocyclooctene-8-carboxylic acid (**122a**, yield 13% and 15%, respectively) by intramolecular cyclization (Scheme 21).

Similar cyclization via $AlCl_3$-catalysed intramolecular Friedel-Crafts acylation provides only a moderate yield (14-21%),[84] in spite of the absence of strain in the bicyclo-nonanone ring system.[85] Other preparations are also known,[86,87] *e.g.* from benzylcyclo-hexanone with methyllithium,[88] from unsaturated enol silyl esters with ceric ammonium nitrate,[89,90] from alkenes by methylsulphite cyclization[91] and by carbocationic cyclization of unsaturated bromoimines.[92]



Scheme 21

To improve the yield of **122a**, we started from dimethyl 4-*trans*-phenylcyclohexane-*cis*-1,2-dicarboxylate (**121a**). Cyclization with polyphosphoric acid (PPA) at elevated temperature yielded a mixture of the isomeric esters **123a** and **123b** in a ratio of 1:5. In contrast, the cyclization of dimethyl 4*t*-phenylcyclohexane-1*r*,2*t*-dicarboxylate (**121b**) gave the esters **123a** and **123b** in a ratio of 5:4 (the yield of **123a,b** was 54%). Consequently, as a result of the transformation **121b**→**123a,b** with PPA, the 30% yield of **123a** isolated from the mixture **123a,b** by column chromatography proved to be sufficient to permit further reactions. We presume that in the cyclization, the 2-carboxy groups, which are axial in the ground state,

come close to the phenyl group by ring inversion and **121a** and **121b** partly isomerize to form the products **123a,b**. After separation of the isomers, the structures were established by NMR spectroscopy. The esters **123a,b** were hydrolysed and the acids **124a,b** were characterized by NMR and for **122a** also by X-ray analysis (Figure 1). The oxo group of **123a** was reduced by the Wolff-Kishner method to afford a mixture of *cis*- and *trans*-5,6,7,8,9,10-hexahydro-5,8-methanobenzocyclooctene-8-carboxylic acids (**124a,b**). With hydrazine, the oxocarboxylic acid **122a** was cyclized to the tetracyclic methanobenzocycloocta[9,8-*c*,d]pyridazinone (**125**).

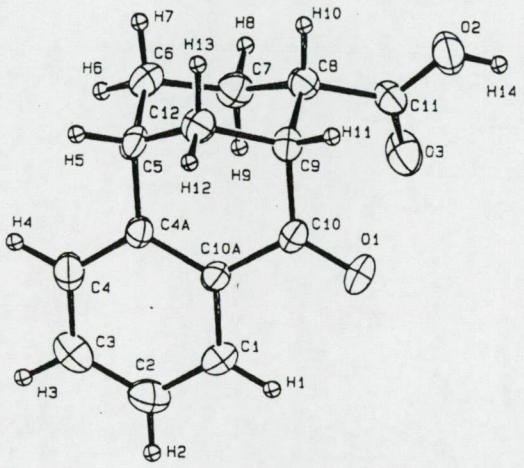


Figure 1

X-ray structure of compound **122a**

In consequence of the strong steric hindrance between the α-axial COOR group and the skeleton, no boat conformation of ring *C* is possible (Scheme 21, **122a**, *i*). For a chair conformation and a *cis* configuration (Scheme 21, **122a**, *ii*), the axial H-8 is in a *trans*-diaxial position with one of the neighbouring H-7 atoms, and the correspondingly large coupling[37] appears in the ^{1}H NMR spectra of one each of the acid and ester isomers; for **122a** and **123a**, the H-8 signal is a triplet of doublets split by 13.3, 4.1 and 4.1 Hz.

As the H-8 multiplet of **122b** and **123b** does not exhibit a large splitting, the chair conformation of ring *C* is also preferred for the *trans* isomers; hence, the COOR group is axial and the equatorial H-8 has no diaxial (*i.e.* large vicinal) coupling (Scheme 21). The spectral data on the reduced products **124a,b** confirm the above structures. For C-7 and C-11, the chemical shifts hardly differ from those measured for **122a,b** and **123a,b**.

For the isomeric pairs **122a,b** and **123a,b**, the shifts of C-6, C-8 and C-11 differ significantly due to the strong steric hindrance between the axial 8-COOR group and H-6_{ax} and H-11_{ax} in the *trans* isomers. For **124a,b**, only the shift difference for C-6 and C-11 is significant; that for C-8 is significantly smaller ($\Delta\delta$C-8 = 1.3 ppm). The explanation lies in the strong steric hindrance between the *endo* 10-methylene hydrogen and the equatorial 8-COOH group of the *cis* isomer, and therefore the C-8 line is also shifted upfield for the *cis* isomer.

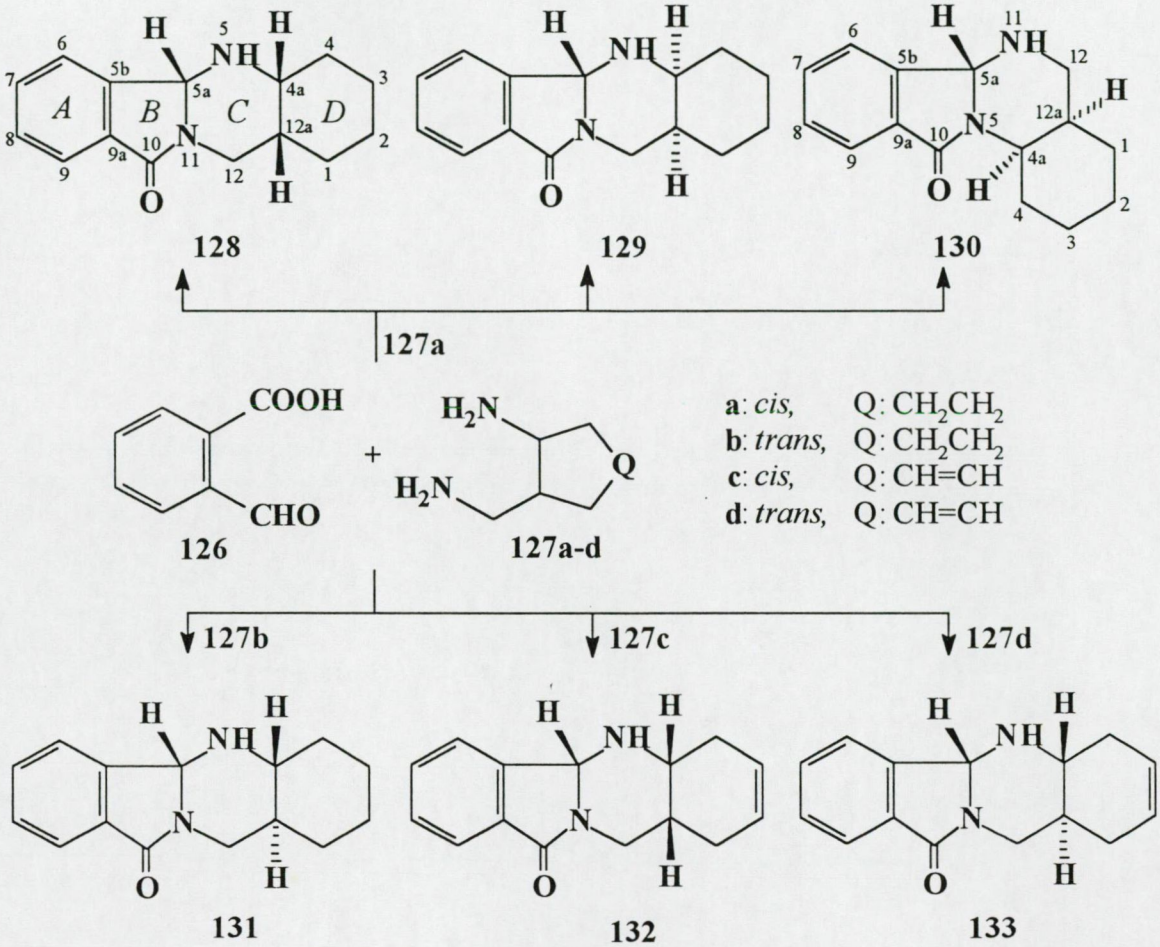
To summarize, the intramolecular cyclization of **121a** with PPA yielded the isomers **123a** and **123b**, which differ in the configuration of C-8; for **123a**, H-5, H-8 and H-9 lie on the same side of ring *C*, while in **123b**, H-5 and H-9 are on the same side and opposite to the hydrogen geminal to the carboxy group. On reduction of the acid **122a**, the isomers **124a** (all-*cis*) and **124b** (5*r*H,8*t*H,9*c*H) are formed in a ratio of 1:2; the epimerization probably takes place via enolization of the 8-CO (carboxy) group.

123a can be prepared from the *trans* ester **121b** more advantageously than from the *cis* ester **121a**, and its 30% yield allows its use as a starting molecule for the synthesis of highly condensed systems. Hence, intermolecular acylation with PPA at an elevated temperature is an appropriate method of obtaining the methanobenzocyclooctene system.

6. Preparation and structure of partially saturated isoindolo[1,2-*b*]- and -[2,1-*a*]quinazolines[6]

2-Carboxybenzaldehyde **126** reacts with cyclic diamines in which one of the functional groups is bound to a cyclohexane/ene ring directly, and the other indirectly through a methylene group. When boiled together in toluene, 2-carboxybenzaldehyde **126** and *cis*-2-amino-1-cyclohexylmethylamine **127a** yielded three products: the linearly condensed **128** and **129** or the angularly-fused **130** (Scheme 22), which were separated by HPLC. After isolation and crystallization, the structures of tetracycles **128-130** were established by NMR spectroscopy. The linearly-fused products **128** and **129** proved to be isoindoloquinazolinones containing an aromatic ring *A*, two condensed hetero rings and one terminal cyclohexane unit. Similar structural isomers have been prepared in the reaction of β-aroylpropionic acid with **127a**.[93]

In the reaction of **126** with *trans*-2-amino-1-cyclohexylmethylamine **127b**, only one product (**131**) was isolated. Likewise, in the reactions of **126** with *cis*- or *trans*-2-amino-



Scheme 22

cyclohex-4-enyl-1-methylamine (**127c** and **127d**), **132** and **133** were obtained. Similarly, to **128** and **129**, these compounds are linearly condensed isoindoloquinazolinones. The spectral data indicate that the formation of these tetracyclic ring systems is straightforward.[6]

The theoretically equally possible linearly or angularly condensed ring systems can be differentiated on the basis of the chemical shifts of the NC*H* and NCH_2 hydrogens. Due to the anisotropic effect of the carbonyl,[59] the former and one of the latter give significantly downfield shifted signals: 4.5 ppm (**130**) instead of 2.6-3.4 ppm (**128**, **129** and **131-133**), and 3.37 (**130**) instead of 4.15-4.43 ppm (**128**, **129** and **131-133**). Consequently, **128**, **129** and **131-133** contain the methylene group vicinal to the amide nitrogen, while in **130** the CH group is bound to this nitrogen atom. Hence, all these new compounds have linearly condensed skeletons, with the exception of **130**, which alone has an angular structure.

The *cis*- or *trans*-condensed cyclohexane rings differ in the carbon shifts: due to the field effect (steric compression shift[63]), the sum of the carbon shifts (ΣδC) is significantly smaller for the *cis* compounds. ΣδC is 184.5 ppm (**128** and **130**) or 188.8 ppm (**129**) for the *cis*-annelated cyclohexanes, and 217.9 ppm for their *trans* counterpart **131** ($\Delta\Sigma\delta C \approx 32.0$ ppm). Similarly, $\Delta\Sigma\delta C$ is 22.6 ppm for the cyclohexene *cis-trans* pair **132** and **133**, and *cis* annelation for **128-130** and **132** is unquestionable.

A further problem is to determine the relative positions of the isoindolone NC*H*N hydrogen and the annelational hydrogens of the partially or wholly saturated quinazoline ring. This was established by means of DNOE measurements.[59, 94]

On saturation of the isoindolone H-5a, responses from H-4a, H-12_{ax} and H-6 were observed for **128** and **131-133**, which proves the steric proximity of the latter atoms to H-5a.

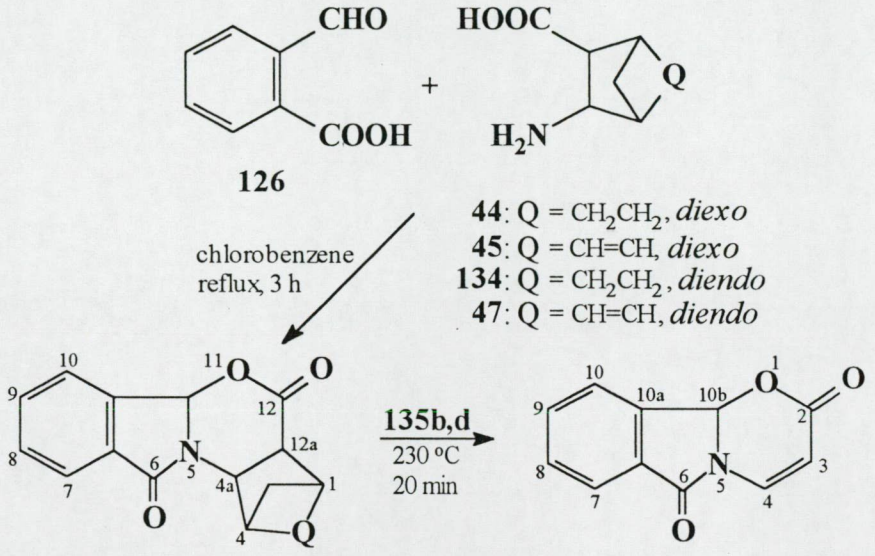
For **129**, the *cis* annelation of the cyclohexane and the vicinity of the methylene group to the amide-*N* was proved above. In the knowledge of the structure of the diastereomer **128**, which contains a *cis*-annelated cyclohexane ring, the only possible structure for **129** that remains is that in which the annelational hydrogens and H-5a are in the *trans* arrangement. In comparison with **128**, the significant field effects on C-5a (5.2 ppm) and (5.8 ppm), due to the steric interaction with the 4-methylene group in the 1,3-diaxial position, are proof of this structure. The corresponding field effects on 2-CH_2 and 4-CH_2 are 4.4 and 5.0 ppm. Consequently, **129** has the relative configuration 4a*R*,5a*S*,12a*R*. The above results support our previous findings:[71, 93, 95] in similar cyclizations, the *cis* or *trans* cyclic 1,3-aminoalcohols or 1,3-diamines always react with retention of the configuration.

7. The first preparation of a heterotricycle, [1,3]oxazino[2,3-*a*]isoindole-2,6-dione, by a retro Diels-Alder method[7]

diendo- and *diexo*-3-Aminobicyclo[2.2.1]hept-5-ene-2-carboxylic acids (**45** and **47**)[38, 21a] were recently used to prepare known and previously unknown heteromonocycles[25] and bicyclic derivatives in retro Diels-Alder (RDA) reactions.[96, 97] The principle of these procedures is the synthesis of the partially saturated parent heterocycles on cyclopentadiene, which is removed from the condensed-skeleton molecules in the final reaction step, resulting in the target compound by introduction of a new double bond. The importance of the RDA reaction

was recently highlighted by its application for the preparation of an enantiomerically pure compound[98] and its study in aqueous solution.[99,100] The present section describes an extension of the scope of this new retrodiene reaction, *i.e.* the application of 2-carboxybenzaldehyde (**126**) for the preparation of fused-skeleton heterotricycles from norbornene amino acids **45** and **47**.

The reactions of 2-carboxybenzaldehyde (**126**) with *diendo*- or *diexo*-3-aminobicyclo[2.2.1]hexane-2-carboxylic acids (**44** and **134**) or *diendo*- or *diexo*-3-aminobicyclo-[2.2.1]hex-5-ene-2-carboxylic acids (**45** and **47**) gave 1,4-methanohexahydro- (**135a,c**) and tetrahydroisoindolo[2,1-*a*][3,1]benzoxazine-6,12-diones (**135b,d**) in moderate yields (45-54%, Scheme 23).



135a: Q = CH_2CH_2, *diexo*; **135c**: Q = CH_2CH_2, *diendo*
135b: Q = CH=CH, *diexo*; **135d**: Q = CH=CH, *diendo*

Scheme 23

In all these compounds, the *diexo*- or *diendo*-norbornene annelational hydrogens and the 1,3-oxazinoisoindoledione NC*H*O hydrogen are in the *cis* arrangement. Similarly as observed earlier for related norbornene-fused 1,3-heterocycles, **135b,d** undergo retrodiene decomposition when heated to their melting points. In these reactions, cyclopentadiene splits off and 2*H*,6*H*-1,3-oxazino[2,3-*a*]isoindole-2,6-dione (**136**) is formed, which can be isolated in 38-42% yield after a simple purification by column chromatography.

Cycloreversion proceeds readily in the retrodiene decomposition, *i.e.* a new double bond is formed between the two carbons of the target molecule if an oxo- or thioxosubstituted heteroaromatic or quasi-heteroaromatic system is formed, *e.g.* pyrimidinone, pyrimidinedione, thioxopyrimidinone or 1,3-oxazine-6-one.[25] The present case is the first example of the formation of a fused tricyclic hetero compound containing two fused partially saturated hetero rings and one aromatic ring in a RDA reaction. The overall structure, but especially the fused 1,3-oxazine-6-one terminal ring in **136**, is very similar to the heteromonocycle 2-aryl-1,3-oxazine-6-ones which were prepared in an analogous way,[22] with the difference that **136** contains no C=N bond between the aryl-substituted carbon and the nitrogen of the six-membered hetero ring. However, in the structure of **136**, a fused aromatic ring is attached to the sp^3 carbon situated between the two hetero atoms, and a benzoyl carbonyl is attached to the bridgehead nitrogen. This structural pattern lends a quasi-aromatic character to the 1,3-oxazine-6-one system produced by the splitting-off of cyclopentadiene, although it is obvious that the formation of this ring system is less favourable than that of the related heteroaromatic monocycles, for in these tricycles the second double bond in the six-membered hetero ring is missing. The energy difference between the heteroaromatic monocyclic and quasi-heteroaromatic tricyclic systems is reflected by the much higher temperature necessary for the formation of **136**.

The present results encourage us to extend the scope of the RDA method in the direction of more complicated hetero compounds in which the electrons required for terminal hetero ring formation are supplied by heteroatoms, *i.e.* the preparation of new polycyclic hetero compounds can be anticipated. Analogous condensed-skeleton heterocyclic compounds similar to the earlier synthesized heteromonocycles may be obtained in RDA reactions from molecules containing electron-rich structural moieties which promote the formation of the new double bond in the product and substitute the second double bond formally required for the aromaticity of the (terminal) hetero ring of the product.

The above method affords an easy synthesis of the previously unknown tricyclic system **136** and illustrates the general scope and importance of formation of a partially unsaturated or aromatic hetero compound via the RDA technique, by building up a heterocyclic molecule on the norbornene derivative prepared from cyclopentadiene and

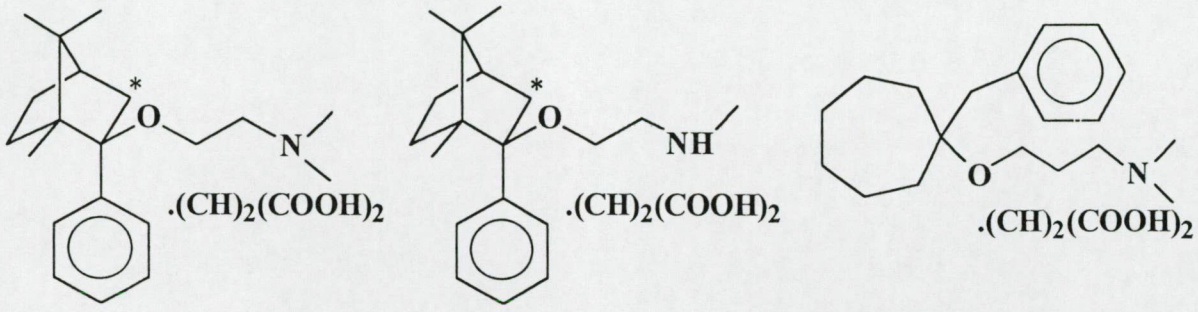
splitting off the cyclopentadiene by heating in the final reaction step. This method does not require the flash vacuum pyrolysis applied in traditional RDA reactions.[101]

The structures of the new compounds **135a-d** and **136** follow straightforwardly from the spectral data.[7] For **135a-d**, the *diexo* or *diendo* annelation of the norbornane/ene unit to the oxazinone ring can be determined via the multiplicity of the annelated hydrogens in positions 4a and 12a.[23, 102] In the *diexo* compounds **135a,b**, as a consequence of the dihedral angles, which are ~90°, the H-1/H-12a and H-4/H-4a interactions are not significant (according to the Karplus relation[37]), and hence the H-4a and H-12a signals are doublets corresponding to the mutual interactions of these hydrogens, while for the *diendo* **135c,d** these signals are double doublets (here the above dihedral angles are ~30°). The relative configuration of C-10b (between the N and O) must also be determined. This is possible by means of NOE measurements.[94, 59] The results of these experiments show the all-*cis* arrangements for H-4a, H-10b and H-12a in all pentacyclic compounds.

8. Comparative bioanalytical study of [^{3}H]deramciclane in dog plasma, using gas chromatography - nitrogen-selective detection (GC-NPD), a new GC-radiochemical detection (GC-RD) technique and liquid scintillation[8]

Deramciclane fumarate (1*R*,2*S*,4*R*)-(-)-*N*,*N*-dimethyl-2[(1,7,7-trimethyl-2-phenylbicyclo(2.2.1)hept-2-yl)oxy]ethaneamine-2-(*E*) butenedioate (1:1) (EGIS-3886, Scheme 24) is a new non-benzodiazepine-type anxiolytic compound[103-106] developed by EGIS Pharmaceuticals Co. For the determination of deramciclane in plasma samples collected in pharmacokinetic studies performed in different species, several different gas chromatography methods have been elaborated. In pharmacokinetic studies on rats, dogs and rabbits, different highly sensitive nitrogen-selective and mass spectrometry detection methods had to be applied because of the low doses and the "first-pass" metabolism, which varied in intensity depending on the species.[107, 108] With these methods, the parent compound and its *N*-demethyl metabolite (EGIS-7056) were selectively determined in plasma samples. In *in vitro* and *in vivo* studies of the metabolism of deramciclane, thin-layer chromatography with digital autoradiographic detection[109] (TLC-DAR), TLC-DAR-FAB-MS-MS[110] coupling and HPLC separation followed by radiochemical detection (HPLC-RD) were applied. In pharmacokinetic studies, liquid scintillation counting (LSC) is frequently used for the determination of drug molecules labelled with different radioisotopes in biological matrices.[111-113] This method allows

determination of the total radioactivity of the sample without separation of the parent compound and its metabolite(s).



[^{3}H]deramciclane fumarate | *N*-demethyl-[^{3}H]deramciclane fumarate | Bencyclane fumarate (internal std. for GC-NPD)

(* denotes the position of ^{3}H-labelling)

Scheme 24

From among the techniques possible in pharmacokinetic and metabolite kinetic studies, radio-gas chromatography (developed in the 1980s) is used relatively infrequently.[114, 115] The HPLC-RD technique offers the advantage of the possibility of metabolite isolation for structure identification at a micropreparative level; the advantage of GC-RD is the high sensitivity.

Comparative bioanalytical investigations were performed by the analysis of plasma samples from beagle dogs treated with [^{3}H]deramciclane. Total radioactivity was determined by LSC, while the plasma levels of [^{3}H]deramciclane and its *N*-demethyl metabolite were determined by capillary gas chromatography, with the applyication of two different detection methods. The previously elaborated GC-NPD bioanalytical method was compared with the new and optimized coupled technique of GC-RD, which allowed selective determination of the radioactive components of the sample. Various pharmacokinetic parameters calculated from the plasma concentration-time curves of the parent compound and its metabolite, measured by different analytical methods, were compared with those obtained by determination of the total radioactivity levels. These results demonstrated the usefulness of this method for the analysis of biological samples.

***In vivo* study phase**

[^{3}H]Deramciclane and unlabelled deramciclane were weighed separately, and then mixed in a ratio of 1:14 to obtain an appropriate radioactive concentration according to the desired limit of detection (approximately 500 pg equiv/ml plasma). The homogeneity and specific activity of the mixture were checked by LSC. The specific activity of the homogenate was 4.80 MBq/mg (287 831 dpm/mg). The radioactive test material was formulated in a hard gelatine capsule. After 7 days of acclimatization and 12 h of fasting, a 1-year-old male beagle dog weighing 9.9 kg was treated with a single 10 mg/kg oral dose of the above [^{3}H]deramciclane formulation.

Blood samples for the pharmacokinetic experiments were taken from the brachial vein with a Vasocan cannula containing 1% heparin in physiological saline solution, or wia a venipuncture: 9 ml of blood was drawn into a glass tube containing 1 ml of anticoagulant (EDTA) solution (the sampling times are presented in Figures 3 and 4). Blood samples were centrifuged at 2000 g for 10 min, and the plasma was separated and stored in a deep-freezer at -20 °C until processing.

Processing of plasma samples

Dog plasma samples stored at -20 °C were allowed to thaw at room temperature and then extracted on a previously washed and activated LiChrolut 200 mg RP-18 solid-phase extraction column, using a previously developed method.[107] Substances to be determined were eluted with 1 ml of 1% (v/v) acetic acid in methanol into an Eppendorf tube. The eluate was next evaporated to dryness in a stream of nitrogen on a lukewarm waterbath. The residue was then dissolved in 50 µl of 2-propanol and transferred into a disposable microvial. Two µl was injected into the gas chromatograph with an autosampler (GC-NPD) or manually (GC-RD).

Determination of [^{3}H]deramciclane and *N*-demethyl-[^{3}H]deramciclane concentration of plasma samples

Quantitative determination of [^{3}H]deramciclane and its main metabolite was performed on the basis of the peak area values recorded by the GC-RD instrument. The equation of the external standard calibration was $\mathbf{Q = 1.111(y-b)/a}$, where **Q** is the ng equiv/ml value of [^{3}H]deramciclane, **a** and **b** are the parameters of the calibration line, **y** is the peak area and

1.111 is a correction factor for the dilution caused by the anticoagulant. In the lack of a reference standard of *N*-demethyl-[^{3}H]deramciclane, its concentration in plasma samples was also calculated with the above equation. In each run, the suitability of the GC-RD system was checked with an extracted plasma sample taken 4 h after treatment (Figure 2).

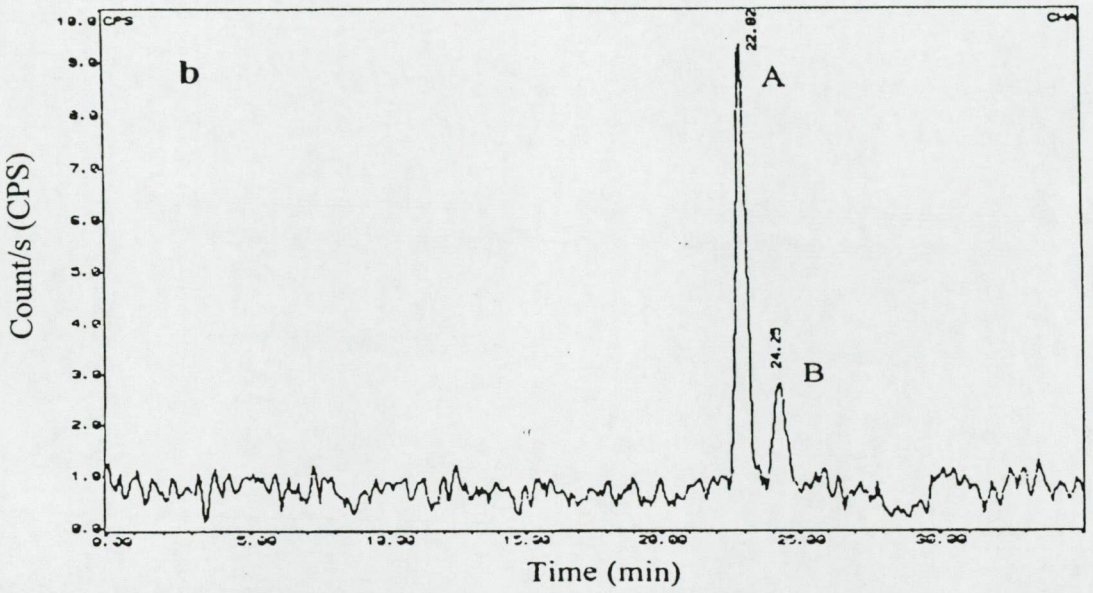


Figure 2. Radio-gas chromatogram of a dog plasma extract; system suitability test. Sampling 4 h after the administration of 10 mg/kg [^{3}H]deramciclane. A: [^{3}H]deramciclane, B: *N*-demethyl-[^{3}H]deramciclane.

The new radio-gas chromatography method

A new, highly sensitive, simple GC-RD method was developed and validated for the selective determination of [^{3}H]deramciclane and *N*-demethyl-[^{3}H]deramciclane. The two compounds were separated on a fused silica capillary column under optimized, programmed temperature and pressure conditions. Tritium-labelled molecules were subjected to reduction at 750 °C in the presence of a platinum catalyst under a stream of hydrogen to yield radioactive methane gas that could be measured with a proportional counter. After a partial validation of the bioanalytical method, the GC-RD system was used to determine the [^{3}H]deramciclane concentration in biological samples.

The method was validated according to the reported consensus.[116] The *linearity* of the calibration curve was investigated in the concentration range 13.71–1000 ng/ml, using spiked

plasma samples. The calibration equation was calculated from the peak area values of [^{3}H]deramciclane with $1/y^2$ weighting, which is common in bioanalytical practice. The 8.9-fold difference between the specific activity of the [^{3}H]deramciclane reference standard and that of the tritiated deramciclane used for the animal treatment was taken into consideration in calculation of the calibration equation. Three calibrators were measured at each concentration level in the wide calibration range in order to determine the linearity, *accuracy* and *precision* of the determination of [^{3}H]deramciclane. The linearity, precision and accuracy were characterized by the coefficient of variation, the RSD and the deviation from the nominal concentration, respectively. The *system suitability* of the radio-gas chromatograph was tested at a concentration of 200 ng/ml, after five parallel injections from a spiked and extracted plasma sample.

The radio-chromatogram (Figure 2) of the extract of dog plasma sample taken 4 h after treatment with a single 10 mg/kg oral dose of [^{3}H]deramciclane revealed the well-separated, symmetrical peaks of the parent compound and its metabolite. The calibration curve exhibited very good linearity for [^{3}H]deramciclane in the concentration range 13.7-1000 ng equiv/ml ($\mathbf{r} = 0.999$). According to the validation results, the lower limit of the quantitation of the GC-RD method was 13.7 ng equiv/ml. The limit of detection was 1 ng equiv/ml; in spite of the low specific activity, this was comparable to the sensitivity of the GC-NPD method.[107]

The inter-day precision and accuracy values for [^{3}H]deramciclane satisfied the acceptance criteria specified for non-radioactive methods. Despite the absence of an appropriate ^{3}H-labelled internal standard, the linearity and reproducibility were acceptable with external standard calibration. The accuracy and precision were on the average 2.35 and 7.19%, respectively. The RSD value obtained during the system suitability test of the GC-RD system was 1.78%.

Pharmacokinetic and statistical analysis

The pharmacokinetic parameters were determined from the plasma concentration-time data by using Siphar/Win validated pharmacokinetic software; the statistical evaluation was performed with Statistica for Windows ver. 4.5 software. With the above GC-RD method, plasma levels of ^{3}H-labelled deramciclane and its *N*-demethyl metabolite were measured in dog (Figure 3) after the administration of a single oral dose of 10 mg/kg. [^{3}H]Deramciclane

displayed relatively fast absorption, and plasma levels could be detected up to 36 h. The pharmacokinetic parameters of *N*-demethyl-[^{3}H]deramciclane were in good agreement with the pharmacokinetic parameters of the same metabolite obtained with the GC-NPD method. The pharmacokinetic curve and the most important pharmacokinetic parameters of [^{3}H]deramciclane were compared with those obtained after previous GC-NPD measurements, and with the non-selective total radioactivity (LSC) data (Table 1, Figure 4).

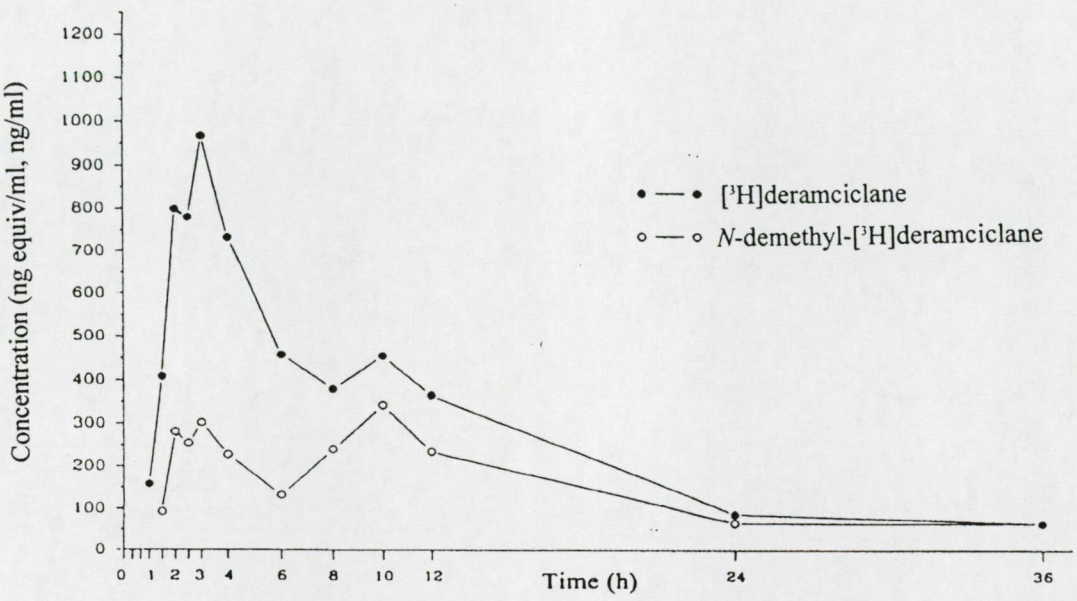


Figure 3. Pharmacokinetic curves of [^{3}H]deramciclane and *N*-demethyl-[^{3}H]deramciclane in dog, determined by GC-RD after the administration of a 10 mg/kg oral dose of [^{3}H]deramciclane.

The pharmacokinetic curves and the calculated pharmacokinetic parameters obtained with the two different, independent detection techniques were closely similar.

The comparison of these results and those obtained by measurement of the total radioactivity in the plasma (LSC) showed that the low plasma concentration ($AUC_{0-\infty}$) of the parent compound was indicative of a very fast and intensive first-pass metabolism. Moreover, the elimination of radioactivity (additional metabolites) ($t^{\beta}_{½}$ = 97.3 h) was very slow as compared with those of the parent compound and the *N*-demethyl metabolite (7.6 h). It could be seen that further metabolites are formed which should be identified for the safety pharmacology.

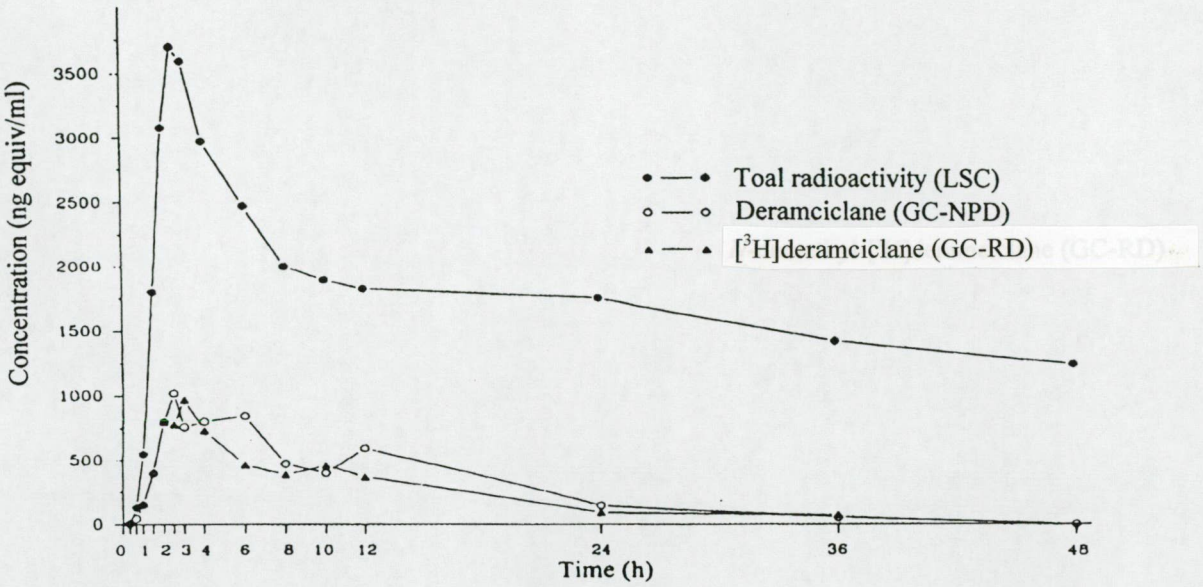


Figure 4. Comparison of the pharmacokinetic curves obtained in dog with various bioanalytical methods

Table 1.

Comparison of the pharmacokinetic parameters of deramciclane calculated from plasma concentration-time data obtained with different bioanalytical methods, after a single oral administration of 10 mg/kg [^{3}H]deramciclane in dog.

Measured moiety	Method	$AUC_{0-\infty}$ (ng*h/ml)	C_{max} (ng/ml)	t_{max} (h)	MRT (h)	$t^{\beta}_{1/2}$ (h)
Deramciclane	GD-NPD	12861,3	1018,3	2,5	9,9	7,68
[^{3}H]Deramciclane	GC-RD	10252,8	967,8	2,9	11,5	7,57
Total radioactivity	LSC	269000,0	3480,0	2,9	138,0	97,30

Summary

Saturated and partly saturated norbornane and norbornene-condensed 1,3-heterocycles were synthesized for pharmacological purposes. In the synthetic work, the resulting isomers were separated by chromatographic methods, which resulted in stereohomogeneous *cis* and *trans*, *exo* and *endo* products.

Thus, by the cyclization of norbornane and norbornene aminoalcohols with imidates, 7 saturated and unsaturated norbornane-fused 1,3-oxazines were prepared and transformed by [2+2] ketene cycloaddition to tetracyclic azetidinones. The isomeric products were separated

and the structures of the adducts were established by means of IR, ^{1}H and ^{13}C NMR spectroscopy. With isothiocyanates, the unsaturated aminoalcohols and the *N*-methyl and *N*-benzyl derivatives were converted into thioureas. The cyclization of the latter gave *diendo*- and *diexo*-2-phenylimino-5,8-methano-3,1-benzothiazines and tricyclic 1,3-oxazinethiones as side-products. Additionally, isothiuronium salts were prepared from the thioureas and cyclized to 1,3-oxazines.

The reactions of 2-aroylcyclopropane and -cyclobutanecarboxylic acids and the corresponding bicyclo[2.2.1]heptane carboxyclic acid with hydrazines yielded cycloalkane-condensed 3(*H*)-pyridazinones and the norbornane *diendo*-fused derivatives. With hydroxylamine, condensed 1,2-oxazine-6-ones were prepared. Transformation of the aroyl cycloalkane-carboxylic acids led to stereoisomeric isobenzofuranones. The partly saturated anthrones and the 2-methyloctahydroanthracene derivatives were also prepared from them. By intramolecular cyclization of 4*t*-phenylcyclohexane-1*r*,2*c*-dicarboxylic acid or its ester in concentrated H_2SO_4, methanocyclooctene isomers were obtained; the oxo group was reduced, to result in *cis* and *trans* methylene-bridged benzocyclooctenes. With hydrazine, tetracyclic pyridazinone derivatives were obtained. The reactions of 2-carboxybenzaldehyde with aminocyclohexenylmethylamines yielded isomeric isoindoloquinazolinones; two linearly *cis*-fused and one angularly *trans*-fused tetracycles were formed.

Hexahydro- and tetrahydroisoindolobenzoxazinediones were prepared by the reactions of 2-carboxybenzaldehyde with norbornane/enecarbocyclic acids. When heated, the norbornene derivatives underwent retrodiene decomposition by splitting off cyclopentadiene to give the 1,3-oxazino[2,3-*a*]isoindole-2,6-dione. This is the first example of the preparation of a condensed heterotricyclic compound in a retro Diels-Alder reaction. The structures of the compounds were established by means of IR, ^{1}H and ^{13}C NMR methods.

A new GC-RD bioanalytical method was used for determination of the plasma level of [^{3}H]deramciclane and its metabolite in dog, after the administration of a single 10 mg/kg oral dose. The pharmacokinetic parameters calculated were compared with those obtained by GC-NPD and total radioactivity (LSC) methods. Comparison of these results demonstrated that deramciclane metabolizes intensively. Moreover, the biological half-lives ($t^{\beta}_{½}$) of the unknown metabolites proved to be an order of magnitude longer than those of the parent compound or the *N*-demethyl metabolite.

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Appendix

Publications related to the thesis